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LOSSES OF ORGANIC SUBSTANCE IN THE SPONTANEOUS HEATING OF ALFALFA HAY[1]

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INTRODUCTION

The annual loss in the United States from fires resulting from the spontaneous heating and ignition of agricultural products is estimated to be approximately $20,000,000, or about 20 percent of the total estimated loss from fires on farms. But this loss, however great, does not take into account the spoilage or complete destruction of farm produce incident to excessive spontaneous heating which stops short of actual fire. In this country, the loss of hay from imperfect curing and subsequent spoilage in the mow or stack probably greatly exceeds the loss of this crop from fire. In harvesting, even under favorable curing conditions, hay seems to lose considerable food value, and when stored properly cured it undergoes some further loss in value incident to the normal "sweating" process in the mow or stack. But in practice it is frequently not possible to harvest hay under favorable conditions, and unless some means is found in manner of storage to offset the previous defects in curing, much more serious deterioration of the hay is sure to follow, with the resulting partial or complete destruction of its food value.

Fleischmann (*5*)[3] has made a very exhaustive investigation of the losses occurring in the harvesting of hay under various conditions. He found that under the most favorable conditions of curing there may be no loss of dry matter at all, and if the drying is not too rapid in uninterrupted sunshine there may be actually a gain in weight, which he ascribes to assimilation. But under less favorable conditions losses occur, and under very unfavorable conditions the loss of dry matter in harvesting may exceed 20 percent.

Similar results are reported by Truninger (*13*). In seven lots of hay which were followed in detail from the time of cutting to the time of storing, there were changes in dry substance ranging from a maximum loss of 11.3 percent to a maximum gain of 1.3 percent. The storage in small stacks of these same lots of hay, whose moisture content ranged from 23.4 to 39.1 percent, resulted in further losses of 2 to 11 percent, or an average of 6 percent for the seven lots.

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[2] The large-scale experiments on the spontaneous heating of alfalfa hay in which this investigation was included were conducted by the Chemical Engineering Research Division, David J. Price, principal engineer in charge, in cooperation with the Food Research Division, F. C. Blanck, principal chemist in charge, under the general supervision of C. A. Browne, supervisor of chemical research, to whom the authors are indebted for helpful suggestions and advice. Associated with the authors in the active conduct of the large-scale experiments were Harry E. Roethe, engineer; L. H. James, senio. bacteriologist; and E. A. Beavens, assistant bacteriologist. Their helpful cooperation in this investigation is acknowledged.

[3] Reference is made by number (italic) to Literature Cited, p. 183.

Fleischmann found that the losses that occurred during harvesting were confined almost exclusively to the nitrogen-free extract and crude fat. Crude fiber suffered no loss, nor did crude protein. But there was always a loss of albumin, which amounted to 25 to 50 percent in slow drying, with a corresponding increase in amido nitrogen.

From an investigation of the fermented hay of his storage experiments and from a comparison of the analyses of samples of normally fermented and of excessively fermented hay, Truninger found that the chief loss occurred in the nitrogen-free extract, the changes in this group ranging from a loss of 40 percent to a slight gain in two cases. Losses were noted also in crude fat. Crude protein and pure protein remained practically unchanged, while in excessive fermentation, loss in amides occurred. The effect on digestible pure protein was very destructive. In only one of a group of excessively fermented samples was the loss less than 50 percent, and in three instances it was complete.

In making brown and black alfalfa by stacking partially wilted alfalfa having an average moisture content of 53.28 percent, Swanson, Call, and Salmon (*12*) found that the total loss of organic matter amounted to nearly 40 percent. The losses in protein, crude fiber, nitrogen-free extract, and ether extract were 32.7 or 36.5, 46.5 or 42.5, 45.4 or 45.8, and 63.6 or 59.2 percent, depending on the method of computation.

In order to secure further information on the extent and nature of the losses in hay substance during the storage of undercured hay, a series of experiments was conducted as one phase of the large-scale spontaneous heating experiments of the Bureau of Chemistry and Soils, at Beltsville, Md. It was thought that such information would be of value in interpreting the causes, the progress, and the results of the spontaneous heating. In 7 of the 10 large-scale experiments, extending from 1929 to 1934, the extent of the losses was determined, and in 4 of these experiments a study was made of the nature of the losses.

EXPERIMENTAL PROCEDURE

An outline is given later of the set-up and progress of the experiments.

The experiments on loss of hay substance are designated by the same numbers that were used in recording the corresponding large-scale spontaneous heating experiments (*6*), and begin with experiment 4 of that series. In all the experiments the hay was stored in an experimental barn at Beltsville. The floor space of this barn covered an area of approximately 26 by 24 feet. The space used for storage was practically 23 by 24 feet, a narrow alleyway of 3 by 24 feet being left on the entrance, or east side of the barn. The hay when stored was in this way exposed on only one side—the east. In one experiment, as indicated, all direct exposure was eliminated.

THE BASKETS OR CONTAINERS FOR HAY SAMPLES

Because of the impossibility of collecting identical samples of hay from selected locations in the mow before and after the heating process, to determine the actual losses in weight suffered by the original hay substance (as stored) during the experiment, it was necessary to

devise some means of confining the samples which would not interfere with the progress of heating and decomposition.

Such containers for the samples would have to be resistant to corrosion, flexible, and porous enough to permit the free passage of moisture and gases, but at the same time not sufficiently open to permit mechanical losses. In the first three experiments of the series, containers, or "baskets", of monel wire cloth were used. A roll of 24-mesh wire cloth, made of 0.013-inch diameter wire, and another roll, 16-mesh, 0.009 inch diameter, were cut into 36- by 30-inch pieces and made up into pillow-shaped containers by crimping, with one end left open for filling. The finished containers were approximately 2 feet long and 18 inches wide when flattened out.

These containers proved to be unsatisfactory because they failed to resist the corrosive action of the fermenting hay. In the four later experiments similar containers were made of Nichrome IV wire cloth, an alloy of 80 percent nickel and 20 percent chromium. This was 16 mesh, 0.013 inch diameter. The finished pillow-shaped baskets were of the same size as the baskets of monel metal. Two such baskets were plated with silver in order to determine the possible resistance of silver to the action of fermenting hay. Although the baskets of Nichrome wire cloth became slightly corroded, they were found to be reasonably satisfactory for the purpose. On the other hand the silver-plated baskets were severely attacked.

COLLECTION AND TREATMENT OF SAMPLES

The procedure followed in collecting the samples to be stored in the mow varied slightly, but in general it was as follows: When preliminary mositure determinations on the hay in the field indicated the moisture content desired for storage, the hay was bunched or cocked. One pile of the best quality hay in the field was selected for the basket samples. This hay was mixed as uniformly as possible before sampling, and in most cases it was quartered and sampled. The desired number of baskets, previously weighed, were then filled with the hay, and the open ends of the baskets were closed by crimping or by the use of wire of similar material. One of these basket samples was treated exactly like the others up to the time of storage, after which it was transferred to the laboratory for further drying and preservation as a standard or reference sample.

Samples corresponding to the basket samples were collected at the same time from the same lot of hay and put into airtight glass fruit jars and preserved in a refrigerator for later determination of moisture. In some cases the moisture content of the hay thus determined was found to differ somewhat from that indicated by losses from the large standard sample from the time of collection to complete drying, but this value was taken as corresponding most closely to the true moisture content of the basket samples.

Immediately after collection the baskets and contents were weighed. Those samples not placed in the mow immediately after weighing were again weighed for purposes of record just before storage. The various baskets were placed in selected locations in the mow after the insertion into the center of each of a thermocouple to be used in following the progress of heating.

At the conclusion of the experiment, during the removal of the hay from the mow, each basket was removed and immediately weighed. The contents were put into airtight glass containers to preserve the final mositure, which was determined later. After removal of the hay, the baskets were weighed again, and their condition was carefully noted.

By this procedure, all the data required for computing the hay losses during storage were obtained. By corrections for ash content of the hay before and after storage in the baskets, a basis was obtained for comparing the losses in toto and by individual constituents or groups of constituents, on the moisture- and ash-free basis. All storage losses were calculated as percentage losses of dry organic substance based on the original dry organic substance, and all the results obtained in the determination of the individual constituents or group of constituents on the moisture-and ash-free basis were corrected finally for these storage losses.

Under the most favorable conditions it is very difficult to secure a number of samples of exactly the same moisture content from a given lot of hay, and to collect samples whose moisture content is truly representative of the moisture content of the hay under investigation. This difficulty is greatly increased when the lot of hay selected is made up of portions of unequal degrees of dryness. Consequently extreme care must be exercised in selecting hay most uniform with respect to quality and degree of curing. Mixing the hay as thoroughly as possible, followed by quartering, before sampling, is practically essential. But even when every available precaution is taken to secure uniform samples, there still remains the possibility of considerable variation in moisture content. It is doubtful whether this possibility can be obviated without chopping the hay and thoroughly mixing it before sampling, and this procedure would involve other difficulties.

In the experiments given in detail later, except the first, a difference of 1 percent in moisture content of the samples stored would involve a difference in dry-substance loss of 1.40 to 1.85 percent. In the first experiment, where the moisture of the samples was very high, an error of 1 percent in moisture would cause a difference of 3 to 4 percent in the results. Fortunately, the difficulty of accurately sampling this freshly cut alfalfa was less because it had not had the opportunity to dry before collection.

METHOD OF DETERMINING MOISTURE

In anticipation of the necessity of making numerous moisture determinations and of the further necessity of drying, without decomposition, large quantities of hay for comparative analysis, a study was made of several methods for determining moisture. Comparison of the results of drying over concentrated sulphuric acid under reduced pressure, of the toluene distillation method, of drying in vacuum (25 to 75 mm of mercury) at various temperatures up to 100° C., and of drying at atmospheric pressure in an air oven at 98° to 100° showed that the drying of samples not exceeding 20 g at 85° in vacuo for approximately 16 hours was equivalent to drying at 100° in vacuo for 5 hours, and was complete. Accordingly, the method selected was drying to constant weight in vacuo at either 85° or 100°.

For the analytical work, subsamples of the large basket samples, in amounts ranging from 100 to 600 g, were dried to constant weight by this method, in suitable vessels of aluminum. This operation also gave the moisture content of the basket samples after undergoing spontaneous heating. In the first two experiments of the series, samples were dried at 85° C., but in subsequent ones the temperature used was 100°. It was found that an average of 52 hours was required for the complete drying at 85° in vacuo of 200 to 600 g of hay containing 50 percent or more of moisture. Less time was required for drying samples of lower moisture content. No evidence of decomposition was noted upon drying in vacuo at 100°, and this temperature appeared to be preferable because of the saving in time. Drying at 100° under atmospheric pressure for more than 1 hour gave excessively high results, and was accompanied by decomposition of the hay, as shown by discoloration of the sample and by progressive losses in weight as the heating was continued.

After complete drying, the subsamples were finely ground in a Wiley mill and kept for the analytical work. Before weighing and subsequent treatment, aliquots of these samples later taken for analysis were again subjected to the method of drying just described.

EXPERIMENTS ON LOSS OF HAY SUBSTANCE DURING STORAGE

EXPERIMENT 4

In experiment 4 the hay was stored on June 2 to 4, 1931, and removed from the barn on July 6 to 8, 1931. It consisted of 6,670 pounds of dry timothy (*Phleum praterise* L.)[4] containing 10.1 percent of moisture, up to a height of 4½ feet from the floor; 14,900 pounds of undercured alfalfa (*Medicago sativa* L.), first cutting, having an average moisture content of 55.7 percent, over an area of 10 feet by 10 feet in a central area of the mow, up to a height of 12½ feet; and 39,130 pounds of cured alfalfa, having an average moisture content of 28.5 percent, which covered and surrounded the undercured section to a height of 16½ feet from the floor.

Eleven baskets of monel wire cloth were filled on June 3 with green alfalfa immediately after cutting, from the dairy farm at Beltsville. The baskets and contents were weighed at once. Baskets 1 to 10 were for storage, and basket S (4) was for use as the standard reference sample. Smaller samples of the green alfalfa were collected in air-tight fruit jars for later determination of the moisture content of the corresponding basket samples. This moisture content was determined as 75.42 percent.

Basket S (4), the standard, was left in the sun for approximately 16 hours of the time from cutting until 3 days later, when the drying was continued in the laboratory. The moisture content of the standard as indicated by total losses during this treatment was 75.50 percent.

The basket samples 1 to 9 were not stored in the mow until the day after cutting, and basket 10 not until 2 days after, but these were sunned for several hours on the day of cutting and kept indoors, well ventilated, the remainder of the time until stored. Table 1 shows the locations of the baskets in the mow, the conditions of storage, and the resultant losses of hay substance.

[4] Dry timothy or cured clover was used in experiments 4, 5, 6, and 8 to vary the set-up of the alfalfa experiments or to supplement a limited supply of alfalfa.

TABLE 1.—*Loss of organic substance in the spontaneous heating of hay*

Basket no.	Location in mow [1]			Temperature			Hay surrounding basket sample	Moisture content of hay surrounding sample		Moisture content of basket sample		Dry matter of sample		Loss [2] $\frac{(A-B)}{(A)}\times 100$	Ash content of dry matter at end of experiment [3]	Dry organic matter lost by 100 g of original dry organic matter
	Distance from east	Distance from south	Height from floor	Maximum: Period after storage	Maximum: °C.	Final		As stored	At end of experiment	As stored	At end of experiment	Original (A)	Final (B)			
	Feet	*Feet*	*Feet*	*Days*		°C.		*Percent*	*Percent*	*Percent*	*Percent*	*Gram*	*Gram*	*Percent*	*Percent*	*Percent*
1	11½	12	7	4	66.5	60	Undercured	60.2	44	70.22	56.79	1,182.51	1,000.77	15.37	12.22	17.55
2	5½	6	9½	7	59	47	Cured	24.2	13	67.74	19.01	1,110.17	906.34	18.36	14.00	22.08
3	8	12	9½	4	69	59	Undercured	58.1	40	67.86	52.13	1,057.76	1,037.30	1.93	12.10	4.34
4	11½	12	9½	11	69	60	do	58.1	40	68.01	54.79	1,135.94	1,096.79	3.45	12.90	6.67
5	14	12	9½	4	67	59	do	58.1	40	66.77	52.28	1,199.99	1,178.12	1.82	11.96	4.07
6	17½	18	9½	6	65	49.5	Cured	24.2	14	68.57	21.76	1,084.62	897.19	17.28	12.00	19.22
7	17½	6	12	7	63	48	do	23.1	13	64.52	23.12	1,016.50	930.06	8.50	11.30	9.93
8	11½	12	12	11	73.5	59	Undercured	48.1	40–46	64.86	43.94	901.51	858.39	4.78	15.40	10.60
9	5½	18	12	4	56.5	42	Cured	25.5	14	64.91	17.06	1,332.77	1,193.96	10.41	11.68	12.18
10	11½	12	13½	10	78	50	do	28.5	13–17	62.90	27.56	1,182.71	1,100.02	6.99	13.56	10.78
EXPERIMENT 5																
1	11½	6	7¼	6	51	30.5	Undercured	31.3	13–15	29.75	15.57	878.25	825.74	5.98	8.62	6.98
2	11½	12	7¼	6	51	31	do	31.3	13–15	29.75	14.42	1,453.92	1,406.30	3.28	8.91	4.62
3	5¾	18	7¼	6	51	30	do	31.3	13–15	29.75	14.72	954.96	911.69	4.53	8.96	5.90
4	17¼	18	7¼	6	50	32.5	do	31.3	13–15	29.75	13.31	1,604.53	932.91	7.13	8.52	8.02
EXPERIMENT 6																
1	5¾	6	7½	5	65.5	12	Undercured	42.8	13.4	42.52	11.65	853.75	932.09	−9.18	11.22	−5.67
2	11½	12	7½	5	67	14	do	42.8	10.7	42.52	13.67	641.27	720.16	−12.30	10.17	−9.99
3	17¼	18	7½	4	66	12	do	42.8	12.8	42.52	13.98	805.98	910.24	−12.94	10.17	−10.61
4	11½	12	10¼	5	67.5	13	Undercured	42.8	17.1	42.52	14.03	588.76	616.79	−4.76	10.75	−1.94
							Dry	16.8	14.5							

EXPERIMENT 7

21	11½	11½	6	6	64	48	Undercured	38.5	25.1	41.12	26.14	373.19	374.84	−0.44	7.49	0.23
22	6	6	6	5	62	49	do	38.5	21.9	41.12	20.87	352.13	354.11	−.56	7.44	.06
28	11½	12½	6	5	59.5	42	do	38.5	25.1	41.12	15.98	436.82	418.00	4.31	7.76	5.22
23	6	6	9	6	61	46	Cured	26.2	12.7	33.44	16.19	457.84	450.40	1.63	7.57	2.37
24	11½	11½	9	6	63	47	do	26.2	17.6	31.83	20.07	490.10	458.40	6.47	7.51	7.12
29	11½	12½	9	6	60	43.5	do	26.2	17.6	28.28	14.49	402.65	409.15	−1.61	7.04	−1.42
30	15½	16	6	8	55	28.5	Undercured	38.5	13.0	41.12	12.92	377.23	324.81	13.90	7.66	14.64

EXPERIMENT 8

1	11½	12	5	20	61	6	Undercured	45	14	46.30	13.78	652.45	602.68	7.63	7.70	8.59
2	11½	12	8	12	65.5	6.5	Cured	30.3	23.6	46.30	22.41	565.46	556.71	1.55	7.23	2.08
3	11½	12	11½	7	63.5	8	Dry	9.8	11.6	46.30	15.97	611.11	613.84	−.45	6.88	−.29

EXPERIMENT 9

1	11½	12	4¼	6	61	21	Cured	29.9	11–14	35.90	11.78	372.75	333.03	10.65	8.35	11.20
							Undercured	43.2								
2	11½	12	6½	4	67.5	27	Undercured	43.2	11	35.90	14.79	461.50	367.26	20.42	9.84	22.20
3	11½	12	8½	7	68	44	Cured	29.9	22	35.90	24.27	412.80	363.88	11.85	10.00	13.97
							Undercured	43.2								
4	5	12	12½	3	47	18	Cured	29.9	10	29.20	9.57	380.20	348.61	8.31	8.22	9.31
5	11½	12	12½	4	67	28.5	do	29.9	10	29.20	14.32	349.75	307.59	12.05	8.61	13.39
6	18	12	12½	5	52	19.5	do	29.9	10	29.20	8.64	431.17	413.40	4.12	8.10	5.04

EXPERIMENT 10

1	11½	18	5	5	54	21	Not salted	32.59	13.06	32.59	13.87	728.03	630.73	13.36	8.33	13.99
3	11½	18	9½	4	54	21	do	35.79	12.67	35.79	13.48	665.54	628.57	5.55	8.50	6.04
5	11½	18	12½	1	49.5	19	do	30.18	11.30	30.18	11.73	431.84	397.66	7.91	7.20	8.05
2	11½	6	5	8	52	21	Salted	37.75	13.06	37.75	15.50	[4] 563.87	[4] 532.73	5.52	[4] 7.48	5.34
4	11½	6	9½	7	56	23	do	34.02	14.32	34.02	15.42	[4] 785.92	[4] 748.49	4.76	[4] 10.32	7.14
6	11½	6	12½	10	53	20.5	do	28.42	12.41	28.42	12.64	[4] 505.69	[4] 438.84	13.22	[4] 7.75	13.87

[1] Horizontal center of mow was 11½ feet from the east side and 12 feet from the south side.
[2] Minus (−) signs indicate gains.
[3] Ash content of original dry matter in standard samples: S (4), 9.89 percent; S (5), 7.63 percent; S (6), 8.28 percent; S (7), 6.87 percent; S (8), 6.73 percent; S (9),7.78 percent; S (9)c, 7.21 percent; S (10) 1, for baskets 1 and 2, 7.66 percent; S (10) 3, for baskets 3 and 4, 8.02 percent; S (10) 5, for baskets 5 and 6, 7.06 percent.
[4] Corrected for salt content.

The maximum temperature in this experiment, 78° C., was reached on June 15 in one of the baskets which had been placed in cured alfalfa just above the center of the horizontal junction of the undercured and cured alfalfa. The temperature at this point had dropped to 71° on June 26, on which date the three next highest temperatures in the mow were 68°, 63.5°, and 61.5°.

The baskets remained in the mow until removal of the hay at the conclusion of the experiment, or 32 to 34 days. The maximum and final temperatures in the respective baskets are recorded in table 1. The drop in temperature from maximum to final was in all cases rather irregular but gradual.

At the conclusion of the experiment the settled height of the mow was 10 feet at the center, 11½ feet on the east side, and 12¾ feet on the west side. The condition of the hay was noted as it was removed from the barn. The central section, which consisted of the originally undercured alfalfa, was damp and brown. Some of it was very dark brown and of very inferior quality. The originally cured hay was light brown except in localities directly above the undercured section, particularly above the center of this section, where it was dark brown. Where the cured and undercured had been in contact considerable mold was found, but otherwise the hay was generally free from mold.

The condition of the hay in the baskets was similar to that of the hay immediately surrounding the baskets. Of the basket samples located in the undercured section, 1, 3, 4, and 5 were brown, and 8 was dark brown. The hay in basket 10, located in originally cured hay, had been subjected to the dampening effect of the underlying wet hay and was dark brown. The other baskets, which had been placed in cured alfalfa, were light brown.

The metal-cloth baskets stored in the wet sections of the mow were severely corroded, baskets 8 and 10 most severely, so that all the samples in these baskets were considerably contaminated with corroded metal. For this reason the ash as determined is not the true ash content of the decomposed hay. Because of this fact the dry-matter losses given in the table are comparable only where computed to loss of dry organic matter by equal weights of original dry organic matter.

EXPERIMENT 5

Experiment 5 was begun on July 13 to 16, 1931, and concluded on August 20, 1931. The hay stored consisted of 9,490 pounds of dry timothy containing 17.2 percent of moisture, to a height of 5½ feet; 9,850 pounds of undercured alfalfa, second cutting, having a moisture content of 31.3 percent, to a height of 8½ feet; 11,728 pounds of cured alfalfa containing 19.8 percent of moisture, to a height of 13 feet; 3,700 pounds of dry timothy, containing 16.1 percent of moisture, to a height of 15 feet.

On July 14 four baskets of monel wire cloth (nos. 1 to 4) were filled with alfalfa hay containing 29.75 percent of moisture, as determined from smaller samples collected from the same lot of hay. On air-drying and subsequent removal of remaining moisture, the standard sample S (5), collected along with the storage samples, lost 33.02 percent of moisture, a figure higher than the 29.75-percent moisture value. Baskets 1 to 4 were placed in their respective positions in the mow immediately after weighing (table 1).

The maximum temperature in the mow, 56° C., was reached on July 19 to 20, at a location 5¾ feet from the east side, 6 feet from the south side, and 8½ feet from the floor, at the junction of undercured and cured alfalfa. The temperatures recorded by the thermocouples in the four baskets were practically the same throughout the experiment, dropping gradually but irregularly from the maximum to the final recorded temperatures.

At the conclusion of the experiment the mow had settled to a height of 11 feet. The hay removed from the barn was found to be in good condition. It was dry and light brown except for the timothy on top, some of the alfalfa underneath this, and the timothy on the bottom. The hay was free from mold except for a few small spots about 4½ to 5 feet from the floor (representing alfalfa stored with 31.3 percent moisture). Basket samples 1, 2, and 3 were light brown; sample 4 was brown.

The metal baskets did not show any marked deterioration resulting from the mild activity in this experiment.

EXPERIMENT 6

The hay in this experiment was stored on August 25 to 26, 1931, and left in the barn until April 12 to 14, 1932. It consisted of 9,515 pounds of dry timothy, containing 17.4 percent of moisture, to a height of 3½ feet from the floor; approximately 1,700 pounds of dry alfalfa, containing 16.8 percent of moisture, to a height of 4½ feet; 24,890 pounds of undercured alfalfa, third cutting, containing 42.8 percent of moisture, to a height of 10¼ feet; approximately 9,600 pounds of dry alfalfa (from experiment 5) containing 16.8 percent of moisture, to a height of 16 feet.

On August 25 five monel wire-cloth baskets were filled with undercured alfalfa hay, third cutting, from a field in the neighborhood of Beltsville. Baskets 1 to 4 were for storage; the fifth basket, S (6), was the standard sample. Samples collected for moisture determinations showed a moisture content of 42.52 percent for the hay in the baskets. Air-drying followed by oven-drying of the standard indicated a moisture content of 40.43 percent.

The highest temperature in the mow, 68° C., was reached on August 29 in undercured alfalfa at a point 5¾ feet from the east side, 6 feet from the north side, and 6 feet from the floor.

The maximum and final temperatures reached in the baskets are recorded in table 1. As judged by the temperature records, all activity had ceased long before the final reading of temperature. In fact, within 34 days after the date of storage, the temperatures in the baskets had fallen from the maxima to 50.5° C. (basket 1), 42.5° (basket 2), 50.5° (basket 3), and 49.5° (basket 4).

The final settled height of the mow was 12 feet at the center. The hay as removed was all very dry, and most of the originally undercured hay was spoiled and of extremely poor quality. Considerable mold was found, especially in the neighborhood of basket 4. The basket samples were all dark brown and dry. The metal baskets were badly corroded, and the samples were contaminated with considerable dust and particles from the corroded baskets.

EXPERIMENT 7

For the purpose of comparison with the alfalfa experiments, clover was used in experiment 7. The hay was stored on June 7 to 10, 1932, and was removed from the barn on August 9 to 10, 1932. The mow was filled as follows: Cured clover containing an average of 31.7 percent of moisture, to a height of 3½ feet; undercured clover containing 38.5 percent of moisture, in an oval section extending diagonally northwest to southeast approximately 10 feet from the center of the mow in each direction and having a width of 8 feet, to a height of 8 feet from the floor; cured clover containing 30.6 percent of moisture surrounding the oval section of undercured clover to its 8-foot height; cured clover having 26.2 percent of moisture, from the 8-foot level to a height of 14 feet from the floor; and ryegrass containing 15.7 percent of moisture, to a height of 16½ feet from the floor. The total hay stored amounted to 31,867 pounds of cured clover (average moisture, 28.2 percent), 5,480 pounds of undercured clover (38.5 percent moisture), and 2,250 pounds of ryegrass (15.7 percent moisture).

On June 8 four baskets of Nichrome wire cloth (samples 21, 22, 23, and 24), two silver-plated Nichrome baskets (samples 28 and 29), and one pure silk bag with numerous small holes cut through it (sample 30) were filled with the undercured clover selected for storage. An additional basket, S (7), was filled from the same lot of clover and reserved as the standard sample. Samples of the same clover showed a moisture content of 41.12 percent. When air-dried and oven-dried, the standard, S (7), suffered a total loss of 37.40 percent.

The highest temperature recorded during the experiment, 64° C., was reached on June 15 at two locations; one was 11½ feet from the east side, 11½ feet from the south side, and 4½ feet from the floor, in undercured clover; the other was 7 feet from the east side, 7 feet from the south side, and 5½ feet from the floor, at the junction of undercured and cured clover.

As will be noted in table 1, the range of temperature within the several baskets did not vary greatly. No thermocouple was placed in the sample in the silk bag, and the temperatures reported for this sample are those of the nearest thermocouple in the mow.

The hay was again weighed as it was removed from the mow at the conclusion of the experiment. The originally cured clover weighed 22,920 pounds (moisture, 11.4 percent); the originally undercured clover, 3,710 pounds (14.8 percent of moisture); and the ryegrass, 1,720 pounds(10.6 percent of moisture). At the time of removal the mow was 11½ feet high at the center, 12 feet on the west side, and 11 feet on the east side.

With the exception of a few isolated spots of mold, the hay after storage was of good quality. That from the interior of the mow was slightly brown. The contents of baskets 21, 22, and 28 were brown; those of baskets 23, 24, and 29 were light brown.

The recorded weights and moisture contents of the hay as placed in the barn and as removed indicated the following approximate dry-matter losses during the experiment: For undercured clover, 6.24 percent; for cured clover, 11.23 percent; for ryegrass, 18.90 percent; for total hay, 11.15 percent. These losses include mechanical losses due to the very dry and shattered condition of the hay as removed.

EXPERIMENT 8

The hay was stored on September 7 to 12, 1932, and removed from the barn on April 11 to 13, 1933. It consisted of cured clover containing 11.5 percent of moisture over the entire storage space, to a height of 4 feet; undercured alfalfa, third cutting, containing 45 percent of moisture, in a central section of the mow, 14 by 14 feet, to a height of 6 feet; cured alfalfa containing 30.3 percent of moisture, over the undercured section, to a height of 10½ feet; dry alfalfa containing 9.8 percent of moisture, over the cured alfalfa of the central area, to a height of 13¾ feet; cured clover containing 11.5 percent of moisture, surrounding the central section, from the 4-foot level to a height of 8 feet; dry alfalfa containing 9.8 percent of moisture, surrounding the central section, from the 8-foot level to a height of 13¾ feet; and cured clover containing 11.5 percent of moisture, covering the entire area of the mow, to a height of 16½ feet from the floor.

The total hay stored was 12,250 pounds of clover, 10,690 pounds of dry alfalfa, 8,590 pounds of cured alfalfa, and 3,790 pounds of undercured alfalfa.

In this experiment and in experiments 9 and 10, the hay was stored not upon the floor of the barn but upon a false bottom, or openwork supports, by means of which the hay was elevated several inches from the barn floor. It was anticipated that in this manner increased aeration of the mow could be accomplished when desired. This false bottom was provided with floor vents, which were left open a part of the time that the experiments were in progress.

On September 8, 1932, four baskets of Nichrome wire cloth were filled with undercured alfalfa hay containing 46.30 percent of moisture, as determined on other samples of the same hay collected at the same time. The baskets and contents were weighed at once. Basket 1 was placed in the mow on the same day; baskets 2 and 3 on the next day. The fourth basket, S (8), was reserved as standard. When air-dried and finally oven-dried, this sample underwent a total loss in weight of 44.04 percent.

The maximum temperature in this experiment, 66.5° C., was reached on September 21, at the thermocouple in basket 2. The three baskets remained in the mow over 7 months, and at the end of this time their temperatures had fallen from maximum to the final temperatures given in table 1. But in the first 2 months the temperatures had dropped to approximately 20°.

At the conclusion of the experiment the mow had settled to a height of 13½ feet at its center. The weights of the hay as it was removed from the barn were: Total clover, 11,050 pounds (average moisture content, 10.9 percent); cured alfalfa and dried alfalfa, 17,280 pounds (average moisture content, 12.6 percent); originally undercured alfalfa, 2,210 pounds (12.1 percent moisture).

The condition of the hay as removed at the conclusion of the experiment varied considerably. Most of the alfalfa hay was brown; the originally undercured hay was dark brown. The hay in the baskets was brown. Considerable mold was also observed, especially in the central section.

The weights of total dry matter stored and total dry matter at the conclusion of the experiment indicated the following approximate losses, including mechanical losses: For clover, 9.18 percent; for dry

and cured alfalfa, 3.44 percent; for undercured alfalfa, 6.85 percent; for total hay in the mow, 5.87 percent.

EXPERIMENT 9

In experiment 9 the hay was stored from June 5 to 13, 1933, and removed from the barn on October 10 to 11, 1933. The hay consisted of cured alfalfa, first cutting, containing 29.9 percent of moisture, on the bottom of the mow to a height of 4¼ feet; undercured alfalfa, first cutting, containing 43.2 percent of moisture, to a height of 8½ feet over an area of 10 by 10 feet in a central section; and cured alfalfa, containing 29.9 percent of moisture, which surrounded and covered the undercured hay to a height of 16½ feet from the floor, a total of 45,225 pounds of the cured alfalfa and 5,250 pounds of the undercured alfalfa.

On June 6, 1933, three baskets of Nichrome wire cloth (nos. 1 to 3) were filled with undercured alfalfa for storage; a fourth basket, S (9), of the same lot of hay was collected as the standard sample. The moisture content of the storage samples was 35.9 percent, as determined from smaller representative samples. On air-drying and oven-drying, the standard basket lost a total of 40.57 percent of moisture, a figure considerably higher than the value for moisture content taken as representative of the baskets.

On June 9, three additional Nichrome baskets (nos. 4 to 6) were filled with samples of cured alfalfa for storage, and a fourth, S (9) c, was reserved as the standard sample for the cured hay. The moisture content of these samples was 29.20 percent. The total loss of basket S (9)c on drying was 30.15 percent, which is in very close agreement with the value 29.20, taken as the true moisture content.

The maximum temperature, 68° C., was reached on June 13 in one of the baskets located 6 feet from the floor, at the exact center of the junction of cured and undercured hay. From June 14 the temperature throughout the mow fell rapidly until July 5, when the temperature at the same location was 60°.

The temperature range within the several baskets is shown in table 1. The drops from maximum to final temperatures were very irregular and accompanied by frequent intermittent changes up and down.

At the conclusion of the experiment the hay in the mow had settled to a height of 13 feet. Of the total hay removed from the barn, 31,975 pounds was cured alfalfa, containing 10.4 percent moisture, and 3,260 pounds was the originally undercured alfalfa, containing 14.2 percent of moisture.

The following observations were made regarding the condition of the hay as it was removed from the mow. That which had been stored as cured hay over the central section of undercured hay was found to be light brown to a depth of about 2½ feet from the settled top of the mow, with a few spots of white mold. From 3½ feet from the top (location of basket 5) to approximately 6 feet from the top, the brown hay covered a smaller area, but became darker with increase of depth until at approximately the level at which basket 3 was located (7 feet from the top) there was a small area of damp and dark-brown moldy hay. The formerly undercured section of the mow, lying between the settled heights of 6 feet and 3 feet from the floor, was dark brown and quite dry. Basket 2 was located in the center

of this section. At the 3-foot level (lower junction of undercured with cured hay), where basket 1 was located, the hay was dark brown with a great deal of mold. The condition of the hay in the baskets was practically the same as the hay in which these were embedded. Baskets 1, 2, 3, and 5, located in central areas in a direct vertical line from lower to higher levels, were dark brown; baskets 4 and 6, in locations of exclusively cured hay, were light brown.

From the data on the amounts and moisture contents of the hay when stored and when removed from the barn, the following losses in dry matter were estimated: Cured hay, 9.72 percent; undercured hay, 6.21 percent; total hay in mow, 9.42 percent.

EXPERIMENT 10

In experiment 10 the walls of the entire storage space up to a height of 13 feet from the floor were insulated with fiberboard seven-sixteenths of an inch thick, and a double wall of the same insulating material was built from east to west to divide the space into two equal sections with an air space about 4 inches wide between them. This arrangement was designed to provide two comparable storage sections. The one on the south side was to hold undercured alfalfa treated with approximately 1.5 percent of salt; the other, on the north side, was for unsalted alfalfa. As it was brought into the barn, each load of hay was divided by forkfuls as equally as possible between the two sections, the hay in one section being sprinkled with salt as it was spread in the mow.

The hay (alfalfa, first cutting) was stored on June 5 to 12, 1934. It weighed 49,110 pounds, and had an average moisture content of 34.8 percent. Each section was filled to a height of 16 feet.

Six samples of the hay were stored in Nichrome wire-cloth baskets, three in each section of the mow at identical levels and at corresponding locations in the respective sections. These samples were each taken at the exact location selected for depositing the basket. The samples for the salted section were salted previously in the general salting operation. Samples were collected also from the same locations for the determination of the moisture content of each basket. These values are given in table 1. At the location of each basket in the unsalted section, a basket sample was collected as the standard representing the hay of both sections before salting. The baskets placed in the mow are designated in table 1 as baskets 1 to 6, the standard baskets as S (10)1, S (10)3, and S (10)5. On complete drying, the standard samples S (10)1, S (10)3, and S (10)5 lost an apparent moisture content of 31.70 percent, 37.86 percent, and 30.10 percent, respectively, values in fair agreement with the figure given in table 1 for the moisture content of the basket as stored.

The maximum temperature of the unsalted hay in the mow, 56.5° C., was reached on June 17, at a location 12½ feet from the floor, 11½ feet from the east side, and 2 feet from the south side. The maximum temperature in the salted section, 57.5°, was reached on June 15, at a location 12½ feet from the floor, 3 feet from the west side, and 2 feet from the north side, and on June 17, at a location 9½ feet from the floor, 11½ feet from the east side, and 2 feet from the north side.

The maximum and final temperatures reached in the six baskets are recorded in table 1. On July 8, approximately 1 month after the

baskets were placed in the mow, the following temperatures prevailed: 36.5° C. (basket 1), 46.5° (basket 3), 37° (basket 5), 39° (basket 2), 51° (basket 4), and 42° (basket 6). After this date the temperatures rose and fell very irregularly until the final readings were taken.

At the conclusion of the experiment, on October 2 to 3, 1934, the settled height of the sections was 13¼ feet. A total of 34,425 pounds of hay (corrected for salt added), with an average moisture content of 12.1 percent, was removed. The relative weights of the hay removed indicated that nearly 53 percent of the total hay had been stored in the salted section.

The loss of dry matter computed from the weights of the hay when stored and later when removed from the mow amounted to a total of 5.46 percent for the two sections.

THE NATURE OF THE LOSSES OF ORGANIC SUBSTANCE IN SPONTANEOUS HEATING

The nature of the losses of organic substance in four of the experiments (experiments 4, 6, 8, and 9, table 1) was investigated, and the results are given in tables 2 and 3. Experiments 4, 6, and 9 were those in which most of the undercured hay was rendered of little value for feeding purposes, while in experiment 8 the hay was spoiled only locally.

TABLE 2.—*Nature of the losses of organic substance in the spontaneous heating of alfalfa hay in experiments 4, 6, and 8*

EXPERIMENT 4.—COMPOSITION OF MOISTURE- AND ASH-FREE SUBSTANCE

Basket no.	Ether extract	Total sugars	Pentosans by hydrolysis	Pentosans computed from furfural	Lignin	Cellulose	Total organic and ammoniacal nitrogen	Albuminoid nitrogen	Amido nitrogen	Crude protein (NX6.25)	Methoxyl	Storage loss of organic substance[1]
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Percent*	*Per cent*	*Per cent*
S (4)	2.70	3.82	10.43	13.18	10.61	26.01	3.11	1.92	1.19	19.44	2.24	.....
1	2.14	1.06	9.83	13.50	12.32	24.89	2.98	1.91	1.07	18.63	.....	17.55
4	1.59	1.56	8.57	9.07	11.94	25.97	3.16	2.08	1.08	19.75	1.85	6.77
6	1.83	1.13	10.83	13.37	12.72	30.02	2.70	1.96	.74	16.88	2.18	19.22
7	2.59	1.16	9.20	13.33	12.55	30.09	2.84	1.97	.87	17.75	2.14	9.93
8	2.21	.91	5.62	10.39	13.97	25.17	3.33	2.17	1.16	20.81	1.72	10.60
10	2.77	.85	5.72	9.95	15.16	25.25	3.29	2.33	.96	20.56	1.71	10.78

EXPERIMENT 4.—COMPOSITION OF MOISTURE- AND ASH-FREE SUBSTANCE CORRECTED FOR STORAGE LOSS

S (4)	2.70	3.82	10.43	13.18	10.61	26.01	3.11	1.92	1.19	19.44	2.21	.....
1	1.76	.88	8.12	11.12	10.17	20.53	2.46	1.58	.88	15.36	.....	.....
4	1.49	1.45	8.00	8.48	11.14	24.24	2.95	1.94	1.01	18.44	1.72	.....
6	1.48	.91	8.75	10.79	10.28	24.25	2.18	1.58	.60	13.63	1.76	.....
7	2.33	1.04	8.51	12.01	11.31	27.11	2.56	1.77	.79	16.00	1.93	.....
8	1.98	.82	5.00	9.27	12.49	22.50	2.98	1.94	1.04	18.63	1.54	.....
10	2.48	.76	5.10	8.88	13.52	22.52	2.94	2.07	.87	18.38	1.52	.....

[1] Minus (−) signs indicate gains.

TABLE 2.—*Nature of the losses of organic substance in the spontaneous heating of alfalfa hay in experiments 4, 6, and 8*—Continued

EXPERIMENT 6.—COMPOSITION OF MOISTURE- AND ASH-FREE SUBSTANCE

Basket no.	Ether extract	Total sugars	Pentosans by hydrolysis	Pentosans computed from furfural	Lignin	Cellulose	Total organic and ammoniacal nitrogen	Albuminoid nitrogen	Amido nitrogen	Crude protein (NX6.25)	Methoxyl	Storage loss of organic substance
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Percent*	*Per cent*	*Per cent*
S (6)	2.71	3.87	10.92	.....	9.84	23.18	3.53	2.81	0.72	22.06	.....	.....
1	2.28	1.13	8.90	.....	12.99	24.95	3.70	3.00	.70	23.12	.....	−5.67
2	2.58	1.42	7.48	.....	11.70	22.70	3.61	2.69	.92	22.56	.....	−9.99
3	2.00	1.43	6.82	.....	12.86	26.08	3.63	2.83	.80	22.69	.....	−10.61
4	1.94	1.40	7.66	.....	12.55	24.16	3.86	3.28	.58	24.12	.....	−1.93

EXPERIMENT 6.—COMPOSITION OF MOISTURE- AND ASH-FREE SUBSTANCE CORRECTED FOR STORAGE GAIN

S (6)	2.71	3.87	10.92	.....	9.84	23.18	3.53	2.81	0.72	22.06	.....	.....
1	2.41	1.19	9.40	.....	13.73	26.36	3.90	3.17	.73	24.37	.....	.....
2	2.84	1.56	8.25	.....	12.87	24.97	3.97	2.96	1.01	24.81	.....	.....
3	2.21	1.58	7.53	.....	14.22	28.85	4.02	3.13	.89	25.12	.....	.....
4	1.98	1.43	7.81	.....	12.79	24.63	3.93	3.34	.59	24.58	.....	.....

EXPERIMENT 8.—COMPOSITION OF MOISTURE- AND ASH-FREE SUBSTANCE

S (8)	1.99	3.03	13.74	14.28	9.22	19.41	3.00	2.54	0.46	18.75	.....	.....
1	1.16	.30	12.69	14.99	11.76	24.82	3.26	2.60	.66	20.37	.....	8.59
2	1.91	.60	8.83	13.34	12.38	22.96	3.16	2.58	.58	19.75	.....	2.08
3	2.10	.19	10.48	12.84	11.90	22.21	3.09	2.62	.47	19.31	.....	−.29

EXPERIMENT 8.—COMPOSITION OF MOISTURE- AND ASH-FREE SUBSTANCE CORRECTED FOR STORAGE LOSS

S (8)	1.99	3.03	13.74	14.28	9.22	19.41	3.00	2.54	0.46	18.75	.....	.....
1	1.06	.27	11.60	13.70	10.75	22.69	2.98	2.38	.60	18.62	.....	.....
2	1.87	.50	8.65	13.06	12.12	22.48	3.09	2.53	.56	19.31	.....	.....
3	2.11	.19	10.51	12.87	11.93	22.27	3.10	2.63	.47	19.37	.....	.....

TABLE 3.—*Nature of the losses of organic substance in the spontaneous heating of alfalfa hay in experiment 9*

EXPERIMENT 9.—COMPOSITION OF MOISTURE- AND ASH-FREE SUBSTANCE

Basket no.	Ether extract	Total sugars	Alcohol-benzene extract	Alcohol extract	Uronic acid anhydride	Furfural from uronic acids	Total furfural	Furfural from pentosans	Pentosans computed from pentosan furfural	Lignin	Cellulose	Total organic and ammoniacal nitrogen	Albuminoid nitrogen	Amido nitrogen	Crude protein (NX6.25)	Storage loss
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
S (9)	3.47	3.32	12.20	10.85	13.14	2.19	8.57	6.38	10.92	7.71	22.47	3.44	2.81	0.63	21.50	.....
1	3.16	.71	11.64	11.62	12.63	2.10	7.86	5.76	9.86	8.63	22.72	3.89	2.76	1.13	24.31	11.20
2	2.85	.34	7.61	10.53	11.90	1.98	7.74	5.76	9.86	11.92	23.37	3.79	2.92	.87	23.69	22.20

EXPERIMENT 9.—COMPOSITION OF MOISTURE- AND ASH-FREE SUBSTANCE CORRECTED FOR STORAGE LOSS

S (9)	3.47	3.32	12.20	10.85	13.14	2.19	8.57	6.38	10.92	7.71	22.47	3.44	2.81	0.63	21.50	.....
1	2.81	.62	10.34	10.32	11.22	1.87	6.98	5.11	8.78	7.66	20.18	3.45	2.45	1.00	21.56	.....
2	2.22	.27	5.92	8.19	9.25	1.54	6.02	4.48	7.68	9.27	18.18	2.95	2.27	.68	18.44	.....

METHODS OF ANALYSIS

There is great confusion with respect to accurate methods of determining the very complex groups of organic constituents of plant material, and it is difficult to know whether or not the individual determinations, however carefully carried out, represent in all cases definite constituents or groups. If a uniform procedure is followed the results on different materials may or may not be truly comparable, and the difficulty of accurate comparison is greatly increased when the original material has undergone decompositions involving the formation of products which seriously affect the applicability of a given method. When different methods of analysis are followed uncertainty results regarding the relative accuracy of the methods and the correctness of the assumption that by the different methods one is determining the same chemical complex. This is especially true in lignin and cellulose determinations. Indeed, there are serious limitations in practically every commonly accepted method of determining the most important groups of organic constituents.

In view of these difficulties and limitations, the following procedures and methods of analysis were employed in arriving at the results recorded in table 2.

Ether Extract

The finely ground sample (approximately 10 g) was dried in the vacuum oven at 100° C. for 5 hours and then extracted for 20 hours in a Soxhlet extraction apparatus with anhydrous ethyl ether. The extract in the weighed flask was placed first on the steam bath for removal of most of the ether and then dried to constant weight in the oven at 100°.

Total Sugars

After removal of residual ether, the residue from the ether extraction was transferred to a 250-cc volumetric flask and treated according to the methods of the Association of Official Agricultural Chemists for the preparation of the solution and its hydrolysis (*1, p. 281*). Total sugars were then determined on aliquot portions of the hydrolized solution by the micro method of Stiles, Peterson, and Fred (*11*).

Hemicelluloses

Two methods were used in the determination of hemicelluloses, and the results are reported in table 2 as pentosans. The first method is based upon the hydrolysis of the material with hot dilute hydrochloric acid and the determination of the reducing sugar produced. This method is open to the criticism that by it not only are true pentosans and hexosans determined, but that some of the true celluloses also may be hydrolyzed. The second method is essentially a determination of the furfural produced by distillation with 12-percent hydrochloric acid. By this method not only are the pentosans and hexosans determined, but also other substances which yield furfural under similar treatment. Among such possible furfural-yielding substances are included uronic acids (glucuronic and xyluronic acids), certain aldehydes, and lignin.

Hydrolysis With Dilute Acid

The finely divided residue from the alcohol extraction of the sugars, after filtering, washing, and drying, was treated with 150 cc of dilute

hydrochloric acid (2 percent of HCl by weight). After the addition of two drops of capryl alcohol to prevent foaming, the sample was boiled under a reflux condenser for 5 hours, filtered, and washed with small portions of hot water. Filtrate and washings were combined and made up to 250 cc. Aliquot portions of this solution were neutralized with sodium hydroxide, and the reducing sugars were determined by the method outlined by Shaffer and Hartmann (*10*).

Distillation With 12-Percent Hydrochloric Acid

In this method of distillation the hay sample was not pretreated for removal of crude fat and sugars. The procedure followed was essentially the Association of Official Agricultural Chemists' method for pentosans (*1, pp. 284–285*), except that thiobarbituric acid instead of phloroglucin was used as the precipitant for the furfural, as recommended by Dox and Plaisance (*4*).

Lignin and Cellulose

The washed and dried residue from the dilute acid hydrolysis of hemicelluloses was used for the determination of lignin and cellulose. The method followed was the 80-percent sulphuric acid method described by Waksman and Stevens (*14*), except that the method of Shaffer and Hartmann was used for the determination of the reducing sugars derived from cellulose. Reducing sugars multiplied by the factor 0.90 yield the corresponding value for cellulose.

Nitrogen

Total organic and ammoniacal nitrogen, albuminoid nitrogen, and amido nitrogen were determined by the Association of Official Agricultural Chemists method (*1, pp. 21, 279*).

Methoxyl

The methoxyl group was determined by the Zeisel method using the Zeisel and Fanto apparatus (*15*) as modified by Phillips (*9*).

Methods of Analysis for Experiment 9

The slightly different procedures described below were followed in analyzing the samples of experiment 9 (table 3), as this procedure appeared to offer certain distinct advantages over the previous procedures.

Ether Extract

The procedure was varied from that previously described by determining the ether extract by the loss in weight of the dry extracted sample.

Alcohol-Benzene Extract

The dried residue from the ether extraction was extracted in the Soxhlet apparatus for 20 hours with a mixture of 32 parts by volume of 95 percent ethyl alcohol and 68 parts of benzene. After extraction the sample was dried in the air for 2 days and then in the vacuum oven at 100° C. for 5 hours. The difference in weight of the sample before and after extraction was taken as the amount soluble in alcohol-benzene.

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Alcohol Extract

The dried residue from the alcohol-benzene extraction was extracted with 80 percent ethyl alcohol for 20 hours in the Soxhlet apparatus. The amount soluble in alcohol was determined by loss in weight.

Total Sugars

Aliquot portions of the alcohol-benzene and the 80 percent alcohol solutions were hydrolyzed as previously described, and total reducing sugars were determined by the method of Stiles, Peterson, and Fred (*11*).

Lignin and Cellulose

Aliquot portions of the dried residue from the extraction with 80-percent alcohol were first boiled with 2-percent hydrochloric acid under reflux condensers for successive periods of 3, 3, and 2 hours, for the removal of hemicelluloses, protein, and ash. After each extraction the solutions were filtered through Jena crucibles of medium porosity. The residues were washed with hot water, alcohol, and ether, and dried in vacuo to constant weight. Lignin in the residues was determined by the gaseous hydrochloric acid method described by Dore (*3*) and modified by Phillips (*9*). Cellulose was determined by the Norman and Jenkins method (*8*). The results for lignin were corrected for nitrogen and ash determined by the usual methods.

Hemicelluloses

Total furfural produced by distillation with 12 percent hydrochloric acid was determined as previously described, by the Association of Official Agricultural Chemists' method for pentosans (*1, pp. 284–285*), using thiobarbituric acid instead of phloroglucin as precipitating agent for the furfural, as recommended by Dox and Plaisance (*4*). In order to correct this furfural value for furfural derived from uronic acids, the latter acids were determined by a modification of the method of Dickson, Otterson, and Link (*2*). The modification consisted in weighing the carbon dioxide evolved by the action of 12 percent hydrochloric acid on the sample instead of determining it by the titration method. The carbon dioxide evolved was dried by passage through concentrated sulphuric acid and absorbed in weighed U tubes containing ascarite. The weight of the carbon dioxide multiplied by 4 gives the weight of uronic acid anhydride, which divided by 6 gives the weight of furfural produced by uronic acids (*7*). By subtracting this furfural from the total furfural, the furfural derived from pentosans is obtained.

Nitrogen

Total organic and ammoniacal nitrogen, albuminoid nitrogen, and amido nitrogen were determined as previously described (*1, pp. 21, 279*).

DISCUSSION OF RESULTS

In experiment 4 (table 1), the smallest losses of organic substance occurred in the samples stored in the interior of the section of undercured hay (baskets 3, 4, and 5); the greatest losses occurred in baskets

2 and 6, located in cured hay beneath which was also cured hay. The losses in baskets 7 and 9, surrounded by cured hay, were comparatively large. One sample (basket 1), located in undercured hay but having a 4-foot thickness of very dry hay only 2 feet below it, underwent a loss very near the maximum. Further, one sample (basket 10), stored in cured hay in the center of a horizontal plane lying above undercured hay, lost practically the same amount as another sample (basket 8) stored in the undercured hay beneath. The hay in basket 10 was undoubtedly affected by the moisture rising from the underlying damp hay.

In experiment 8, the greatest loss occurred in the sample (basket 1) located in undercured hay, with a smaller loss in the sample located in cured hay and a slight gain in the sample in very dry hay. In this case there was a much thicker section of very dry clover underneath the undercured section, while the samples lying in the cured and dry hay must have been affected by the moisture rising from the undercured hay beneath them.

The results of experiment 9 appear to be diametrically opposite those of experiment 4. In this experiment the sample (basket 2) of undercured hay located in the interior of similar undercured hay suffered the greatest loss, while the losses were less in baskets 1 and 3, placed at the lower and upper junctions of the undercured and cured hay. In the samples of cured hay placed in cured hay, the greatest loss occurred in basket 5, which was directly over the center of the undercured area.

From these observations it is evidently impossible to determine whether samples of undercured hay stored in the mow in similar undercured hay, and consequently in immediate surroundings of high moisture content, will undergo a greater or a smaller loss in organic substance than such undercured hay in surroundings of lower moisture content. And this is not surprising when we take into account the fact of the migration of moisture from warmer to cooler parts of the mow. If the moisture in a mass of hay in one part of the mow is high enough to provide favorable conditions for the development of spontaneous heating, there will be a rise in temperature and the evolution of water vapor. In another part of the mow there may be hay of lower moisture content, perhaps not enough to produce any appreciable heat. Were it not for the possibility of the transference of moisture from one locality in the mow to another, it would be safe to predict that in the latter case there would not be any great decomposition of hay substance, as compared with that in the first instance. But, through the migration of moisture from the damper and warmer locality, exactly the required amount of moisture might accumulate here to provide ideal conditions for the maximum heat production and decomposition of the hay. By further migration of moisture this condition in turn would influence still other parts of the mow. Such transference of moisture would be affected by the ease with which air currents or vapors could flow through the intervening mass of hay. Channels or "flues" in the heating mow would influence the direction of this flow as well as the degree and rapidity of moisture elimination from the heating localities. Under these conditions the moisture content in different parts of the mow would be continually changing throughout the entire progress of the heating as long as temperature variations continued. Consequently, one cannot predict with cer-

tainty in what locality or under what initial local moisture conditions the greatest degree of spontaneous heating will occur or where there will be the greatest loss in value of the hay.

However, viewed from another angle, the results of experiments 4, 8, and 9 appear to be in fairly close agreement. It will be observed that in experiment 4, with one exception (basket 1), the samples of undercured hay (baskets 1, 3, 4, and 5) which were stored in hay of high moisture content and which lost comparatively little moisture, at the end of the experiment had undergone very little loss of organic substance. But in the other samples, located in hay of lower moisture content, the moisture loss was greater and the loss of organic substance was also greater.

In experiment 8, the sample located in the undercured hay, which suffered the greatest loss of organic substance, had dried out to a very low moisture content. The maximum loss in experiment 9, which was in the sample stored in the hay of highest moisture content, was also coincident with the extent of drying.

It is not to be inferred, however, that the decomposition or loss of organic substance was the result of the drying of the hay under experimental conditions. Such drying resulted from the free passage of air through the sample, and the occurrence and progress of decomposition were in large measure dependent on the access of air.

In experiment 10, in which half the mow was salted, it was observed that in both the salted and the unsalted hay the loss of organic substance was least in the samples of hay (baskets 2 and 3) containing the highest percentage of moisture and embedded in surroundings of equal moisture content. Probably it is not significant that the greatest loss in the salted hay was in the basket at the highest level in the mow, while in the unsalted hay the greatest loss was in the basket at the lowest level.

The results of experiment 6 are entirely out of line with those of the other experiments in that instead of losses apparent gains in weight of organic substance were found. It does not seem at all porbable that these apparent gains represent real gains in weight of organic substance. In fact, at the conclusion of the experiment the samples of hay appeared to be badly spoiled, and subsequent analysis showed that they had undergone considerable decomposition and loss of several groups of organic constituents. On the other hand, a close scrutiny of the experimental data and calculations of results failed to disclose any evidence of error that would justify the rejection of the results.

In the discussion of the difficulty of accurate sampling of the hay in the field, the possibility of considerable variation in the moisture content of the samples was pointed out. In this experiment a moisture content of 3 percent less than the 42.52 percent used in the calculations of dry matter would practically wipe out the apparent gains in weight in two of the four samples, but a moisture value of nearly 7 percent less would be required to eliminate the gains in all four samples. Still further variation in moisture content in the same direction would be required to establish losses in weight. While some of the apparent gains in weight could be accounted for by an assumption of error in moisture value, it is not believed that such an error could have been of sufficient magnitude and in the same direction in all the samples to account fully for the results.

However, there is a further possibility of throwing light on the discrepancy. In experiment 4 very small losses occurred in three samples (baskets 3, 4, and 5) of very wet alfalfa stored in the interior section of the mow, where the average moisture content of the hay was 55.7 percent. This section of the mow contained approximately 15,000 pounds of hay. A small error in moisture value inherent in the sampling would have been sufficient to convert the small losses into apparent gains.

The conditions of storage of three samples of experiment 6 (baskets 1, 2, and 3) were very similar to those of the three samples of experiment 4 referred to above. Samples of undercured hay containing 42.52 percent moisture were located in the interior of the section of 25,000 pounds of hay containing 42.8 percent moisture. If the results under these conditions are assumed to be of approximately the order of those of the three samples of experiment 4, it would not be difficult to conceive of similar or even smaller losses here, which through the error in moisture value admittedly possibly, would appear as gains.

The possibility that the losses in experiment 6 were in fact smaller than those in experiment 4 is supported by a comparison of the relative microbial and chemical activity as evidenced by the degree and duration of heat production. Reference to table 1 will show that the maximum temperatures reached in samples 3, 4, and 5 of experiment 4 were 67° and 69° C. and that at the end of 34 days from the beginning of storage, when the experiment was terminated, these temperatures had fallen to 59° and 60°, a decline of 8° to 10°. For samples 1, 2, and 3 of experiment 6, the maximum temperatures were slightly lower, 65.5° to 67°, and at the end of 34 days from the beginning of storage there had been declines in temperature of 15° to 24.5°. Although this experiment was not concluded until more than 6 months later, the temperatures continued to fall gradually to the final temperatures recorded.

Further evidence of minor activity and less loss of organic substance is found in the results of analyses of samples of gas collected in the 34-day interval referred to above. These analyses, not included in this paper, showed far less carbon dioxide content for the samples from experiment 6 than for those from experiment 4, the lower carbon dioxide evolution indicating less microbial and chemical action in the mow.

The results of experiment 7 appear on the whole fairly consistent with the observations regarding the foregoing experiments, but no attempt will be made at this time to correlate the results of this single set-up with clover with those of the several alfalfa experiments.

When organic substance losses, both maximum and average, in the basket samples of the several experiments are compared, it is seen that the greatest losses occurred in experiments 4 and 9. In experiment 4, the losses ranged from 4.07 to 22.08 percent, with an average loss of 11.74 percent. In experiment 9, the losses in undercured samples were 11.20 to 22.20 percent, with an average loss of 15.79 percent. In these two experiments, the general storage conditions in the mow were very similar. Very wet hay was stored in a mow in which the average moisture was comparatively high, namely, 33.2 percent (experiment 4) and 31.3 percent (experiment 9). Consequently, conditions were conducive to excessive losses and decomposition. In experiment 4, approximately 24 percent of the total hay had a high

moisture content (55.7 percent), and this hay was surrounded by hay of average moisture content (nearly 26 percent). In experiment 9, 10.4 percent of the total hay, containing 43.2 percent moisture, was surrounded by hay having an average moisture content of approximately 30 percent.

The maximum losses in experiments 5 and 8, 8.02 and 8.59 percent, respectively, were less than those in experiments 4 and 9. The average loss in experiment 5 was slightly more than 6 percent; in experiment 8, it was less than 3.5 percent. The hay in experiment 5 consisted of almost cured hay (31.3 percent moisture), approximately 28 percent of the total, and well-cured hay (18.2 percent average moisture). The average moisture content of the whole mow was approximately 22 percent. Perhaps the losses in this experiment correspond very closely with those occurring normally in the storage of well-cured hay. In experiment 8, less than 11 percent of the total hay was of high moisture content (45 percent); the remainder of the mow had only 16 percent moisture. It would appear from this experiment that a relatively small proportion of very damp hay, when stored in a mow in which most of the hay is very dry, will dry out rapidly and may not suffer serious losses.

In experiment 7, nearly 14 percent of the hay (clover) contained over 38 percent of moisture, and the remainder contained more than 27 percent. The average for the whole now was nearly 29 percent. But here only one sample (basket 30) showed a loss greater than 7.12 percent, and the average loss for all the baskets was only 4.03 percent. Furthermore, the hay when removed from the barn was of good quality and apparently uninjured.

The fact should be borne in mind, however, that loss in weight alone may not be an exact measure of the loss in feeding value. This is evident in the case of experiments 4 and 9, in which the comparatively large losses in weight are far from sufficient to account for the full extent of the deterioration of the undercured hay.

The hay of experiment 10 when stored had an average moisture content of approximately 35 percent. It was stored under conditions of insulation that precluded the free passage of air into the mow from the sides, while the false bottom, referred to under experiment 8, provided aeration from the bottom. Whatever the relative effects of these opposing influences, the rise of temperature in the mow was restricted, and the hay was not seriously injured. The experiment failed to show any appreciable difference in results due to the use of salt in one section of the mow. The average loss of organic substance in the baskets located in salted hay was quite close to that in the unsalted hay, 8.78 percent as against 9.36 percent.

No relation was evident between length of storage and extent of losses in the several experiments. However, the effect of temperature should be taken into consideration. In only one experiment (experiment 4) was the maximum temperature as high as 78° C. The maximum for the samples of experiment 9 was 68°; the maxima for experiments 7 and 8 were 64° and 65.5°, respectively, and for experiments 5 and 10, 51° and 56°. The average maximum temperature of the samples in experiments 4, 9[5], 7, 8, 5, and 10 was 66.7°, 65.5°, 60.6°, 63.3°, 51°, and 53.1°, respectively. In experiments 4 and 9, in which

[5] Undercured samples.

moisture conditions were apparently more favorable to heat production and in which the greater losses of organic substance occurred, the maximum temperatures reached in the samples were considerably higher than in experiments 7 and 8, and very much higher than in experiments 5 and 10. On the other hand, no effect of temperature differences within the individual samples of any one experiment was evident, but here the results were undoubtedly influenced also by other local conditions.

In the outlines of experiments 7, 8, 9, and 10, values are given for the losses of dry matter computed from the weights of the hay stored and the weights of the same hay at the conclusion of the experiments. The values for hay losses in three of those experiments (experiments 7, 8, and 9) are not comparable with these determined for the samples of undercured hay stored in the metal baskets because the mows were made up of hays of more than one moisture content, some being undercured, some cured, and some very dry, while the undercured hay of the baskets was hay of selected moisture content stored under more or less initially definite local conditions and in no sense representative of the entire mow. On the other hand, in experiment 10 the basket samples were really representative of the hay of the mow in which they were stored. Here the average loss of dry matter for the six basket samples was 8.39 percent, as compared with a loss of 5.46 percent determined by weighing the entire mow.

In order to determine the loss of dry matter due to the spontaneous heating of hay of a given moisture content by weighing the entire mow at the beginning and again at the conclusion of an experiment, it would be necessary to store hay of practically uniform quality and of the same moisture content throughout. In removing very dry hay from the mow a mechanical loss occurs from shattering the hay, particularly the leafy portions, and the carrying away of much of this in dense dust clouds. Such mechanical loss would be included in the total loss.

With respect to the nature of the losses in organic substance, as shown in tables 2 and 3, it will be noted that there was a loss in ether extract, or crude fat, in all samples except two, and in these there were small gains. The losses ranged from approximately 6 to 47 percent. Spectrophotometric examination of several of the ether extracts showed that the loss in crude fat was accompanied by the complete destruction of carotene, the precursor of vitamin A. The significance of this fact is readily apparent, because it is essential that dairy herds have a plentiful supply of this minor constituent of properly cured hay.

The sugars suffered severe loss; in several cases they were almost entirely destroyed. The losses ranged from 59.1 percent to 93.7 percent, indicating a very high degree of active fermentation.

Hemicelluloses as measured by the pentosan value underwent considerable loss—13.9 to 52.1 percent as determined by hydrolysis, and 4.1 to 35.7 percent as determined by the distillation method. As noted before, pentosans determined by the distillation method include uronic acids and other furfural-yielding substances. A correction for uronic acids in the results in table 3 showed an amount equivalent to approximately 25 percent of the total furfural. The greatest losses in pentosans occurred in experiments 4 and 9, the experiments having the greatest total organic substance loss. Cel-

lulose also suffered losses in experiments 4 and 9. The losses ranged from 6.7 to 21.1 percent. One sample showed a slight gain. The results for experiments 6 and 8, on the contrary, show gains.

The results for lignin appear as gains, except in three samples, for which very small losses are recorded.

In the five samples of experiment 4, the only experiment in which this group was determined, methoxyl was lost to the extent of 13.8 to 32.1 percent. As the determination of methoxyl was made on unextracted samples, these results are not strictly comparable with the results for lignin as determined directly.

Total organic and ammoniacal nitrogen suffered losses ranging from 4 to 30 percent in the samples of experiments 4 and 9, except in one sample, which showed a small gain (0.3 percent). With one exception the samples of experiments 6 and 8 showed gains.

The samples of experiments 4 and 9 that showed losses in albuminoid nitrogen slightly exceeded those in which there were gains. The maximum loss was 19 percent; the maximum gain, 8 percent. All samples of experiments 6 and 8 except two appeared to gain albuminoid nitrogen.

The results for amido nitrogen indicate a loss in all samples of experiment 4, a gain in all of experiment 9, and a gain in all but one of experiments 6 and 8.

The significance of these results in their relation to the causes, or effects, of spontaneous heating appears to lie in the fact that spontaneous heating, with the production of temperatures not exceeding 78° C., caused a loss in the fats, the sugars, and the hemicelluloses in all the experiments, and that where storage conditions were favorable for excessive losses, cellulose and crude protein were also lost.

SUMMARY

In a series of seven large-scale storage experiments, six with alfalfa hay and one with clover, the extent of the losses of the organic substance of hay resulting from spontaneous heating was determined, and in four of these the nature of the losses was investigated. The length of storage in these experiments ranged from 1 to 7½ months. The samples of hay investigated contained moisture ranging from approximately 28 to 70 percent. The moisture content of the hay immediately surrounding the samples in the mows was between the limits of approximately 10 and 60 percent, while the average moisture content of the mows ranged from 18 to 35 percent.

The greatest losses in organic substance as determined by loss in weight occurred in those experiments in which hay of very high moisture content was stored in a mow whose average moisture was also high. In these experiments the maximum loss was approximately 22 percent; the minimum, 4 percent; the average, nearly 13 percent. Losses in the individual samples varied with the respective conditions and surroundings in which these were located in the mows, particularly with respect to the free passage of air.

The losses in organic substance were much less when the hay samples approximated normally cured hay in moisture content and were stored in a mow of much lower moisture content. Here the maximum loss was 8 percent, the average about 6 percent.

When a relatively small proportion of the mow was of high moisture content while the remainder was very dry, the losses were also comparatively small for samples of high moisture content, the maximum loss being 8.6 percent, the average loss less than 3.5 percent.

The effect of temperature upon the losses of organic substance was noted. It was found that the greatest losses occurred in experiments in which the moisture conditions were favorable to heat production and in which the highest temperatures were recorded.

The results of the investigation demonstrate the seriousness of the losses in the value of hay resulting from the spontaneous heating of undercured hay, and they show further that loss in weight alone of organic substance does not indicate fully the extent of loss in value, as evidenced by the fact that most of the undercured hay of those mows in which the maximum loss was 22 percent had deteriorated into hay of very inferior quality.

The one experiment designed to show the effect of salting upon spontaneous heating failed to show any material difference in hay substance loss due to the use of approximately 1.5 percent of salt.

Investigation showed that the losses of organic substance incurred under these storage conditions involved definitely and chiefly the fats, the sugars, and the hemicellulose group. Where the extent of losses was greatest, cellulose and crude protein also had been attacked. Lignin apparently suffered no loss.

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KEEPING QUALITY OF SUGAR BEETS AS INFLUENCED BY GROWTH AND NUTRITIONAL FACTORS[1]

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INTRODUCTION

The prevention of losses in storage of sugar beets (*Beta vulgaris* L.) during the period between harvest and processing in the factory constitutes a major problem of the sugar-beet industry. For example, a large western company, basing its estimates on the difference between the gross sugar purchased, as determined from tonnages and sucrose percentages, and the gross sugar in the beets actually sliced, placed the shrinkage in sugar for one campaign at more than $5,000,000. Some portion of this loss occurs as a result of respiration of the beet itself, but a large part is occasioned by the activities of organisms that cause decay of the root tissues during the period in which the sugar beets are being stored in piles.

In the western United States, except in California, where the harvest in general is gaged to factory capacity, deliveries of sugar beets in excess of the immediate factory needs are dumped into storage piles. Storage of the roots permits harvesting to proceed during favorable fall weather and assures the factory a supply of beets for continuous operation.

Heavy losses in storage have been noted that could be traced to such practices as storing frozen beets or allowing excessive amounts of soil or debris to be dumped in the piles. Careless handling of roots in the field or in piling may so injure the roots as to promote fungus invasion. Tompkins and Nuckols (*5*)[3] have given data on hook injury and other wounds made during harvesting that are contributing factors to excessive rotting. They have also shown (*6*) that the upper crown tissues are more resistant to attack by rot-producing organisms than are the tissues exposed if the beets are topped one-half inch or more below the leaf-scar level, indicating the desirability of emphasis on proper topping. Portions of the root tissues die and fungus invasion follows if excessive drying of the roots takes place either before storage or in the piles. Pack (*3*) has shown that wetting the roots in storage piles is a desirable practice for western areas. Heavy losses have been experienced in years of unseasonably high air temperature. Artschwager and Starrett (*1*), in experiments in which sugar beets were stored at a number of controlled temperatures and humidities within the range of conditions likely to be encountered in factory storage piles, have shown that wounded beets either do not form a

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[3] Reference is made by number (italic) to Literature Cited, p. 198.

protective wound periderm or form it so slowly as to be unable by this means to ward off attacking organisms.

Preventive measures against storage losses have been of a general nature, attention being given largely to the environmental conditions of storage. The purpose of this paper is to show that certain environmental factors concerned with nutrition and growth of the roots have a marked effect upon the keeping quality of sound roots.

The experiments reported in this paper were initiated because of observations made in 1931 on some immature sugar-beet roots that had been grown in nutrient cultures deficient in phosphorus. It was noted by L. M. Pultz [4] and the writer that these roots, in contrast with roots of similar age but grown with an adequate phosphorus supply, were extremely susceptible to invasion by *Phoma betae* (Oud.) Frank. Reports in the literature have called attention to the rotting of roots in the field as a result of nutritional deficiencies (*4*), but the nutritional or other growth conditions in their effects upon the keeping qualities of stored sugar beets have not, so far as the writer knows, received attention.

EXPERIMENTAL RESULTS

INFLUENCE OF SUPERPHOSPHATE AND OF A COMPLETE FERTILIZER ON KEEPING QUALITY

Methods

Two sugar-beet fields located near Hyrum and Payson, Utah, which were known to be deficient in available phosphates, were chosen for growing the roots used in the storage experiments in 1932. Heavy applications were employed to secure maximum responses and to establish strong differences in quality between the roots receiving fertilizers and the unfertilized roots. Treble superphosphate (46 percent) was applied at the rate of 250 pounds per acre and a complete fertilizer (4–12–4) at the rate of 575 pounds per acre. The fertilizers were applied to strips of land running the full length of the field, an unfertilized strip being left between those fertilized. To avoid injury to the seedlings from the heavy applications, one-half of the fertilizer was broadcast and harrowed in before the seed was sown, the remainder being applied in the row with the seed.

In the 1933 experiment, at Hyrum, Utah, smaller amounts, more nearly corresponding with commercial applications, were used in similarly laid-out plots. The fertilized plots received 150 pounds of superphosphate (46 percent) or 350 pounds of the complete fertilizer (4–12–4) per acre. Cultivation and irrigation of the beets followed standard commercial practices for the area.

In the fall representative roots were selected from each of the three plots receiving the different treatments, namely, (1) phosphate fertilizer, (2) complete fertilizer, and (3) no fertilizer. The largest and smallest roots were discarded as not being representative. The beets selected were topped accurately at the bases of the lowest leaf scars, thus exposing comparable root tissues. After the desired number of roots had been secured from each treatment they were sorted into 10 approximately uniform groups. Nine of these were stored, and the tenth was divided into five samples and used at once in analyses to determine initial sucrose percentages and apparent purity coefficients. In the experiment of 1932 at Hyrum each of the 10 groups from each

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treatment consisted of 100 roots; in the experiment of 1932 at Payson each consisted of 75 roots; and in the experiment of 1933 at Hyrum each consisted of 50 roots.

Nine of the groups taken as representative of each treatment were put into coarse-meshed onion sacks for storage in commercial storage piles.[5] In this manner the identity of the experimental beets was retained, and the circulation of air and gaseous byproducts of respiration was unhampered. Three replicates of each treatment were placed on each of three levels in the storage piles, the various samples being so randomized that the storage-pile exposures were equalized so far as possible for the roots originating from the three conditions of field growth.

In the 1932 experiments final analyses for sucrose percentages and apparent purity coefficients were made on a single 20-beet sample taken at random from each replicate of each field treatment. In the 1933 experiment (Hyrum) two 10-beet samples chosen at random from each replicate of each field treatment were used. This method was adopted in preference to using a single 20-beet sample from each replicate because many of the roots were badly decayed. In the statistical reduction of data, the analysis of variance method (*2*) has been used except in those cases where it was appropriate to determine the standard error of each mean. The values given in Fisher's *t* table for a probability of 0.05 have been used in all experiments to determine the differences required for significance.

Experiment at Hyrum, Utah, 1932

In 1931 the field at Hyrum had grown a mediocre crop of beets, immediately following several years of alfalfa. In the 1932 experiment superphosphate stimulated growth to some extent during the seedling stage, but the greatest stimulation was obtained from the complete fertilizer. This difference between the two fertilizers soon disappeared, presumably as the soil nitrates became more available with the advent of warmer weather. Seedlings in plots receiving complete fertilizer were very vigorous, the loss after thinning being only 3 percent; seedlings in plots receiving superphosphate were considerably less vigorous, the loss amounting to 11 percent; while in the unfertilized strips 16 percent of the seedlings died after thinning. The unfertilized beets remained unthrifty throughout the season, showing symptoms typical of phosphorus deficiency. Many plants in these plots later became affected with *Phoma betae*, which accounted for another 15-percent loss of beets. Symptoms of nutritional deficiencies and disease were entirely absent in beets receiving the fertilizer treatments. At harvest, beets in the unfertilized strips yielded 8.22 tons per acre, those in the superphosphate-fertilized strips 13.49 tons per acre. Beets treated with the complete fertilizer yielded 14.61 tons per acre.

These beets were harvested on October 25 and were placed in storage at Lewiston the following day, where they remained until December 10. Three replicates of each treatment were lost in fluming out the commercial beets, so only six were available from which to take the final data. The record of roots decayed and of changes in sugar content that occurred during storage is summarized in table 1.

[5] Facilities for storage were provided in the storage piles of the Amalgamated Sugar Co. at Lewiston, Utah, and of the Utah-Idaho Sugar Co. at Payson, Utah.

TABLE 1.—*Effect of nutritional conditions on keeping quality of sugar-beet roots stored 45 days under commercial conditions at Hyrum, Utah, 1932*

[Initial readings and change during storage based on comparable samples. Results given as averages of 6 100-beet samples]

Treatment	Weight of 100 roots		Sucrose expressed as percent		Apparent purity coefficient		Indicated available sugar [1]		Roots invaded by organisms
	Initial	Change during storage	Initial	Change during storage [2]	Initial	Change during storage [2]	Initial	Change during storage [2]	
	Pounds	*Percent*					*Pounds*	*Percent*	*Percent*
Superphosphate (46 percent), 250 pounds per acre	134.83	−6.64	16.86	+0.45	88.46	+1.04	20.11	−3.04	14.17
Complete fertilizer (4-12-4), 575 pounds per acre	142.79	−7.79	16.52	+.15	89.00	−1.11	20.99	−8.13	8.33
Unfertilized	84.50	−8.63	16.42	+.04	89.79	−1.21	12.46	−9.63	38.00
Difference required for significance		.83		.19		1.39		1.26	5.68

[1] Obtained by computing for each sample and then averaging. Hence, the value given differs slightly from product of means shown in this table.
[2] See footnote 6.

In the experiment carried on at Hyrum in 1932, 38 percent of the roots in the samples taken from the unfertilized plots were invaded by rot-producing organisms. In contrast to this, only 8.33 percent of the roots which had received a heavy dosage of complete fertilizer during their growing period were invaded by organisms, and 14.17 percent of those that had been fertilized with superphosphate. The difference between the two lots receiving fertilizers reaches the value required for significance. The resistance to rot-producing organisms of roots fertilized with either of the commercial fertilizers was superior to that of the unfertilized roots; while roots receiving complete fertilizer were apparently more resistant than those receiving only treble superphosphate.

The roots receiving superphosphate during their growing period lost only 3.04 percent of their indicated available sugar, whereas the roots grown in complete fertilizer and the unfertilized roots lost 8.13 percent and 9.63 percent, respectively. Because of the limited invasion by organisms causing decay, it is probable that these losses came about as a result of respiration. Statistical analysis places the difference required for significance as to sugar loss at 1.26 percent.[6]

EXPERIMENT AT PAYSON, UTAH, 1932

Because of poor growing conditions, the beets in the experiment at Payson in 1932 did not develop normally. Responses to fertilizer treatments were less striking than at Hyrum, owing primarily to a refractory soil condition and a shortage of soil moisture. The unfertilized beets developed symptoms of both phosphorus and nitrogen deficiency, and invasion by *Phoma betae* caused heavy losses during the growing period. The phosphorus requirements of the crop apparently were satisfied by the fertilizers applied, there being no

[6] The assumption is made that the initial sampling of beets to determine sucrose percentage at the start of the storage period was adequate to establish a mean with negligible error from which to determine change when the beets were stored. The changes in sucrose percentage and in indicated available sugar in tables 1, 2, and 3 have been determined in accordance with that assumption and are expressed as differences between average initial and average final readings. Differences required for significance for these attributes have been determined from the standard deviation of the stored samples.

leaf necrosis nor invasion by *P. betae* to indicate a deficiency. However, the beets in the plots receiving the superphosphate fertilizer showed evidence of a nitrogen deficiency and reached maturity sometime before those beets that received the complete fertilizer. The beets were harvested on October 29 and were placed in the commercial storage pile at Payson the following day, where they remained until December 8. The results obtained on the keeping quality of these beets during storage are summarized in table 2.

TABLE 2.—*Effect of nutritional conditions on keeping quality of sugar beets stored 40 days under commercial conditions at Payson, Utah, 1932*

[Initial readings and change during storage based on comparable samples. Results given as averages of 9 75 beet samples]

Treatment	Weight of 100 roots		Sucrose expressed as percent		Apparent purity coefficient		Indicated available sugar [1]		Roots invaded by organisms
	Initial	Change during storage	Initial	Change during storage [2]	Initial	Change during storage [2]	Initial	Change during storage [2]	
	Pounds	*Percent*					*Pounds*	*Percent*	*Percent*
Superphosphate (46 percent), 250 pounds per acre	88.78	−8.76	16.88	+1.17	92.86	−1.94	13.91	−4.47	34.67
Complete fertilizer (4-12-4), 575 pounds per acre	87.75	−8.42	14.54	+.63	86.99	+.06	11.10	−4.40	44.44
Unfertilized	56.31	−8.19	16.50	+1.06	94.31	−3.95	8.76	−6.37	60.00
Difference required for significance		.77		.22		1.13		1.57	8.08

[1] Obtained by computing for each sample and then averaging. Hence the value given differs slightly from product of means shown in this table.
[2] See footnote 6, p. 188.

Isolations were made of organisms from discolored tissues of beets in the 1932 experiments at Hyrum and at Payson. When examined following the period of storage the beets did not show sufficient tissue involvement to permit trustworthy data being obtained by measuring the depth of penetration or by paring off the invaded tissues for weighings. Judgment was made as to soundness of the stored roots and as to invasion by organisms by observation of the individual roots. One hundred and eleven of the roots judged as invaded by organisms were subsequently cultured. Only 3 of these roots failed to yield organisms; 10 yielded *Penicillium* spp., and 98 yielded cultures of other organisms known to be associated with rotting of beet roots, distributed as follows: 65 cultures of *Phoma betae*, 26 of *Fusarium* spp., and 7 of phycomycetous fungi. The crown tissues and basal portions of the taproot were the chief avenues of fungus entrance; about 15 percent of the diseased beets showed invasion at the sides, 50 percent invasion of the basal portions, and 35 percent crown invasions. None of the various fungus species or groups indicated were restricted in their occurrence to any particular portion of the root or to any specific field treatment.

EXPERIMENT AT HYRUM, UTAH, 1933

Sugar-beet roots for the storage experiment at Hyrum in 1933 were grown in the same vicinity as those for the 1932 experiment. Fertilizer applications were inadequate to counteract the soil deficiencies; consequently beets of rather poor quality were produced in the

fertilized plots as well as in the unfertilized plots. Foliage symptoms of phosphorus deficiency were prevalent in the unfertilized beets throughout the summer and fall and developed to some extent in the fertilized beets just prior to harvest on October 22. Symptoms of deficiency were less severe in the beets that received superphosphate than in those that received the complete fertilizer. Root rot caused by the invasion of *Phoma betae* was again prevalent in the unfertilized beets and resulted in a heavy loss.

Unseasonably high temperatures, a comparatively long storage period, and poor quality of beets operated to bring about rather heavy losses in this experiment. A high percentage of the roots throughout the treatments showed such advanced invasion by rot-producing organisms that it was possible to pare out and weigh rotted portions. Each sample of 50 beets was divided into 5 comparable 10-beet groups on the basis of size of root. Three of these aliquots were used in obtaining data on decayed tissues. The two remaining aliquots served as material for the final sugar and purity determinations (table 3).

TABLE 3.—*Effect of superphosphate and complete fertilizer treatments on keeping quality of sugar-beet roots stored 75 days under commercial conditions at Hyrum, Utah, 1933*

Treatment	Weight of 50 roots		Sucrose expressed as percent [1]		Apparent purity coefficient		Indicated available sugar [1] [2]		Decayed tissue [4]	
	Initial	Change during storage	Initial	Change during storage [3]	Initial	Change during storage [3]	Initial	Change during storage [3]		
	Lb.	*Pct.*					*Lb.*	*Pct.*	*Lb.*	*Pct.*
Superphosphate (46 percent), 150 pounds per acre	53.80	−5.44	19.48	−0.35	97.13	−7.11	10.19	−12.73	2.10	4.23
Complete fertilizer (4–12–4), 350 pounds per acre	54.57	−6.96	18.63	−1.01	96.82	−8.79	9.84	−19.88	3.14	6.18
Unfertilized	30.14	−7.20	19.00	−3.04	96.89	−18.03	5.66	−34.94	4.80	17.48
Difference required for significance	-------	1.59	-------	1.51	-------	5.26	-------	9.26	1.23	3.42

[1] Based upon 2 10-beet samples from each replicate.
[2] Obtained by computing for each sample and then averaging. Hence the value given differs slightly from product of means shown in this table.
[3] See footnote 6, p. 188.
[4] Calculated for the 50-beet sample from the average of three 10-beet samples.

This experiment afforded an opportunity for observing the beneficial effects of corrective fertilization of sugar beets during their growth period on the keeping quality of the roots during a long period of storage. The conditions were conducive to heavy losses from fungus invasion in addition to those resulting from respiration of the stored roots. It will be noted that 17.48 percent, by weight, of the root tissues of beets from the unfertilized plots was destroyed by rot-producing organisms during the 75-day storage period, as compared with only 4.23 percent in roots that came from the phosphate-fertilized plots and 6.18 percent of roots that received the complete fertilizer. A comparison of the results of the 1932 experiments at Hyrum and Payson with these results shows conclusively that the destruction of tissues by rotting organisms in all cases had a direct bearing on the loss of sucrose during storage. In the 1933

experiment, in which root decay was most pronounced, samples of roots that received superphosphate fertilizer during growth lost 12.73 percent of sugar (indicated available) during storage, and samples taken from the plots receiving complete fertilizer lost 19.88 percent, as compared with a loss of 34.94 percent in the unfertilized beets. The difference between the two fertilizer treatments does not reach the value required for significance, but in both cases the fertilized roots showed a significantly lower loss of sugar than those not fertilized. In this experiment the loss of sucrose due to respiration of the root tissues is obscured by the heavy losses caused by rot-producing organisms.

EFFECT OF FERTILIZER, MANURE, AND SOIL-MOISTURE TREATMENTS IN THE FIELD ON KEEPING QUALITY AT HYDE PARK, UTAH, 1932

Field experiments for the control of "dry rot" or "late blight" of sugar beets were carried on near Hyde Park in 1932. Roots from these plots were used to test the influence of the nutrition and of the water supply furnished the growing beet on invasion and decay by organisms during storage. The soil used was extremely low in fertility, and thus it was possible to study the effect of a complete fertilizer as well as of the individual elements in relation to keeping quality of sugar beets. As shown in table 4, four inorganic-fertilizer treatments and an untreated check were used alone and in combination with barnyard manure under two conditions of soil moisture. The manure was applied in the spring before the ground was plowed. The commercial fertilizers, containing 20 percent of plant food, were applied at a uniform rate of 400 pounds per acre at seeding time. Rainfall was meager during the summer, so the crop was almost entirely dependent upon irrigation for moisture. The plots designated as having inadequate soil moisture were irrigated on June 28, July 21, and August 23, as contrasted with the plots receiving adequate water, which were irrigated every 2 weeks, beginning June 14 and continuing to September 15.

The results on the keeping quality of beets as measured by invasion and extent of decay caused by rotting organisms are summarized in table 4. Because of the variability in size and weight of roots taken from the various field treatments, the data have been presented as actual weight of decayed tissue and as percentages of tissue decayed.

Considering first the tests without manure applications, the data obtained are in accord with the results of the preceding experiments in that the application of superphosphate or complete fertilizer during the growth of the beet materially improved the keeping quality of the roots during storage, judgment being based upon the extent of decay occurring. Nitrogen (as ammonium sulphate) applied to this nitrogen-deficient soil produced beets that were markedly superior in keeping quality to those from the unfertilized plots. Beets fertilized with potassium sulphate were likewise superior to the unfertilized roots. Apparently these soils were so deficient in fertility that the addition of any fertilizer produced a favorable response. Shortage of water during the growth period decidedly increased the amount of decay, in some cases almost nullifying the beneficial effects of the fertilizer.

TABLE 4.—*Decay of root tissues as influenced by fertility and soil-moisture conditions during growth of sugar beets near Hyde Park, Utah, 1932*

[Data given as averages of seven 10-beet samples stored 170 days in root cellar]

Fertilizer treatment		Weight of sample	Weight of decayed tissue	Proportion of sample decayed	Invaded beets per sample
Commercial	Organic				
		Pounds	*Pounds*	*Percent*	*Number*
ADEQUATE SOIL MOISTURE DURING GROWING SEASON					
Superphosphate	Manure	23.07	1.32	5.72	6.7
	None	19.82	1.36	6.86	8.3
Complete (4-12-4)	Manure	19.86	.71	3.58	6.4
	None	16.21	2.21	13.63	8.9
Potassium sulphate	Manure	19.46	1.86	9.56	8.1
	None	13.64	2.07	15.18	8.3
Ammonium sulphate	Manure	21.85	.32	1.46	4.3
	None	18.14	1.39	7.66	7.6
None	Manure	18.18	2.25	12.38	7.1
	None	13.04	3.61	27.68	9.3
INADEQUATE SOIL MOISTURE DURING GROWING SEASON					
Superphosphate	Manure	16.18	1.64	10.14	7.0
	None	10.86	4.71	43.37	9.4
Complete (4-12-4)	Manure	14.54	1.61	11.07	7.0
	None	9.39	5.29	56.34	9.7
Potassium sulphate	Manure	14.86	2.18	14.67	8.9
	None	7.46	3.07	41.15	9.9
Ammonium sulphate	Manure	16.11	1.50	9.31	6.3
	None	10.21	4.43	43.39	9.9
None	Manure	13.11	3.00	22.88	8.9
	None	9.04	5.68	62.83	9.7
Difference required for significance			.91	5.97	1.3

The use of barnyard manure alone as a fertilizer produced beets of superior keeping quality as compared with beets from the unfertilized plots. When used in conjunction with a commercial fertilizer, manure significantly increased the beneficial effects except when used with superphosphate and potassium sulphate in the series having adequate soil moisture. In the case of the latter, the value approaches significance. It seems fair to conclude that adequate phosphate nutrition of the beets is a prime essential to keeping quality, and that the manure applications function by influencing phosphate and other nutritional conditions as well as by exerting a very definite effect upon the water relations. From a comparison of the two moisture series, it is evident that barnyard manure did much toward counteracting the deleterious effects of inadequate soil moisture during the growing period on the keeping quality of the roots during storage. The necessity for an adequate supply of soil moisture during growth and its influence in the promotion of storage quality seem clearly demonstrated by contrasting the results of the two irrigation series.

Isolations of the associated organisms were made from decayed tissues of 16 beets selected at random from each of the 20 treatments. A section of each root was given surface disinfection with a mercury-bichloride solution (1:1,000) and then rinsed several times in distilled water. Isolations of the organisms were made from newly invaded tissues, in duplicate, on potato-dextrose agar. Of the 320 roots from which isolations were made, *Phoma betae* was obtained in pure culture from 224 beets and was found associated with *Penicillium* spp. in 19 others. Eleven roots had been invaded by *Fusarium* spp. and four

by *Pythium* spp. The remainder of the cultures were sterile, owing either to deep penetration of the disinfectant into the tissues or to culturing from uninvaded tissues. The distribution of the several fungus forms was approximately uniform throughout the various treatments, there being no association between treatment and type of organism involved.

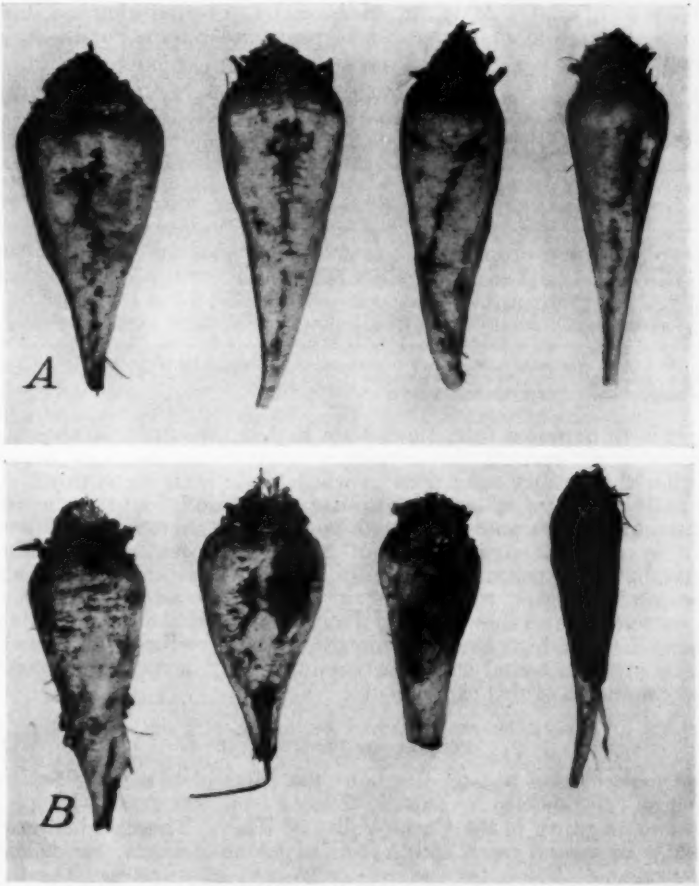

FIGURE 1.—Roots after 45 days' storage in root cellar at Salt Lake City, Utah, in 1933: *A*, Roots from plots well-nourished with respect to phosphorus; *B*, roots from plots deficient in phosphate nutriments.

ROOT CELLAR STORAGE TEST IN 1933 AT SALT LAKE CITY, UTAH

Further evidence of the influence of phosphorus on resistance of root tissues to invasion by organisms was obtained in a test in which beets were stored in a root cellar at Salt Lake City, Utah, for 45 days in 1933 (fig. 1). The soil in which these beets were grown, near Hyde Park, was too deficient in available phosphates to maintain growth without phosphate fertilizer. Beets in treatments designated

as relatively high nitrogen were taken from border rows and were characterized by extremely heavy top growth, while those in treatments designated as relatively low nitrogen were taken from adjacent rows. Foliage symptoms of phosphorus deficiency developed during September in beets that received only 150 pounds of superphosphate (46 percent) per acre, as contrasted with a healthy condition of beets that were fertilized with 275 pounds per acre of superphosphate (46 percent). The data on invasion by rot-producing organisms during storage of roots that were grown under these conditions are shown in table 5.

TABLE 5.—*Influence of available phosphorus and nitrogen on resistance of beets to invasion by rot-producing organisms during storage*

[Data taken after 45 days of storage in root cellar at Salt Lake City, Utah, 1933] [1]

Treatment	Beets stored	Beets invaded	
	Number	*Number*	*Percent*
Relatively high nitrogen:			
Superphosphate, 275 pounds	57	[2] 3	5.3
Superphosphate, 150 pounds	74	23	31.1
Relatively low nitrogen:			
Superphosphate, 275 pounds	64	[2] 6	9.4
Superphosphate, 150 pounds	75	24	32.0

[1] The writer is indebted to L. M. Pultz, formerly associate physiologist, Division of Sugar Plant Investigations, for taking the data presented in this table.
[2] Incipient stages of tip invasion.

It is to be noted from these tests that the quantity of phosphate available determined the keeping quality of the beets in storage. Under the fertility conditions in which these beets were grown, 150 pounds per acre of superphosphate was insufficient to maintain optimum growth and to promote good keeping quality of the roots during storage. Applications of 275 pounds per acre of superphosphate (46 percent) materially reduced the incidence of decay, the only evidence of rotting being incipient tip invasion of a few roots. Available nitrates showed little, if any, effect. However, it is evident that relatively high available nitrate during growth of the beets did not show detrimental effect on keeping quality during storage under the conditions of this experiment.

EFFECT OF PHOSPHATE FERTILIZATION ON RESISTANCE OF BEET TISSUES TO INVASION BY PHOMA BETAE

An inoculation test to determine the effect of phosphate fertilization on resistance to invasion by *Phoma betae* was made with sugar-beet roots grown in the Cache Valley of Utah. The field had grown alfalfa for several years, and the soil, of dolomitic origin, was deficient in available phosphates and relatively high in nitrates. The fertilized roots were taken from a plot that had received 150 pounds per acre of 46-percent superphosphate at planting time. An adjacent untreated strip in the field served as a source of unfertilized roots for the inoculation test. When harvested, the fertilized roots ranged in weight from 175 to 325 g each and the unfertilized roots from 50 to 120 g each.

The roots were surface-sterilized with mercury bichloride solution (1 : 1,000), rinsed in sterile distilled water, and dried with sterile gauze. They were then cut uniformly at the base of the lowest leaf scars to expose uniform crown tissues for inoculation and were cut

near the basal region of the taproot for the tip inoculations. The organism with the agar substratum was placed on the freshly cut surfaces, each root receiving the same quantity of inoculum. Moistened sterile cotton was placed over the inoculated areas, the roots being wrapped in waxed paper to prevent desiccation. Each treatment consisted of 10 inoculations of individual roots.

Tompkins and Pack (7) have shown that strains of *Phoma betae* differed in rate of advance into beet tissues and also react differently with respect to temperature. In these tests two isolates of *Phoma betae*, both originating from storage rotted beets, were selected on the basis of their rate of growth on artificial media. Isolate no. 63 was a rapid grower, producing a heavy mat of aerial mycelium, while isolate no. 31 grew somewhat slower and was characterized by sparse aerial mycelium. Inoculated roots were stored under two temperature conditions: 4° to 6° C., representing a good temperature for storage; and 18° to 22°, temperatures that are occasionally encountered in storing beets.

Observations on inception of decay were made after 9 days of storage. At that time invasion in roots stored at temperatures of 4° to 6° C. could not be measured, the only evidence of organism invasion being incipient decay in the case of the unfertilized roots. In the roots stored at 18° to 22°, after 9 days the decay had advanced to such an extent that it was possible to determine by measurement the depth of invasion. Similar measurements could not be made in the roots stored at 4° to 6° until 18 days after inoculation. The results in this test, expressed as depths of invasion in millimeters, are given in table 6, as averages for the 10-beet groups.

TABLE 6.—*Specific influence of phosphate fertilizer on resistance of sugar-beet tissues to invasion of Phoma betae under different conditions of storage*

Fertilizer treatment	Isolate no.	Crown-tissue inoculations		Basal root-tissue inoculations	
		Depth invaded [1]	$\frac{D}{S.E._D}$	Depth invaded [1]	$\frac{D}{S.E._D}$
ROOTS STORED 9 DAYS AT 18° TO 22° C.					
		Mm		*Mm*	
Superphosphate	31	6.2± 0.3590		7.1± 0.7520	
None	31	12.2± 1.0924		14.8± 1.1813	
Difference		6.0± 1.1489	[2] 5.22	7.7± 1.4000	[2] 5.50
Superphosphate	63	3.1± .6227		3.8± .7860	
None	63	6.6± .5416		12.3± .9776	
Difference		3.5± .8252	[2] 4.24	8.5± 1.2530	[2] 6.78
ROOTS STORED 18 DAYS AT 4° TO 6° C.					
Superphosphate	31	5.0± 0.4956		5.9± 0.5667	
None	31	7.9± .4819		8.6± .5416	
Difference		2.9± .6814	[2] 4.26	2.7± .7839	[2] 3.44
Superphosphate	63	1.6± .1633		5.5± .9690	
None	63	8.2± .5333		10.8± .6541	
Difference		6.6± .5578	[2] 11.83	5.0± 1.1705	[2] 4.27

[1] Average of 10 inoculations.
[2] 2.2 necessary for significance.

It is evident from the results of these inoculation tests that beets that had been well nourished with respect to phosphorus during their growing period were more resistant to invasion by *Phoma betae* than beets that were grown under conditions of a phosphorus deficiency. Statistical analysis of data shows a significant difference in the rate of invasion of the organism under all conditions of storage.

Depths of invasion into crown tissues and root tissues when compared are found to show statistically significant differences in three instances, when isolate no. 63 was used. In the storage test at 4° to 6° C., basal-root tissues of both fertilized and unfertilized roots were invaded to greater depths than were crown tissues. Similar results were obtained with unfertilized roots stored at 18° to 22°, differences with fertilized roots not reaching significance. In the case of the other pathogen used, isolate no. 31, significant differences were not obtained. From this test, however, some basis is given for the conclusion that crown tissues of the beet are more resistant to invasion than basal-root tissues.

DISCUSSION AND CONCLUSIONS

The foregoing investigations furnish evidence that the keeping quality of sugar beets is definitely related to the conditions under which the beets are grown. Nutritional factors and water supply of the growing plant produce effects that carry over when the beet roots are stored.

In general, giving the growing plant enough phosphate to prevent the appearance of marked symptoms of phosphate deficiency, at least early in the season, had not only an influence upon production but also upon the keeping quality of the roots. The effects secured seemed dependent upon the degree to which the phosphorus requirements of the plant were satisfied. The improvement in keeping quality may have been twofold, (1) in decreasing the invasion and decay caused by rot-producing organisms and (2) in reducing the loss of sucrose as a result of the physiological processes accompanying respiration. On the latter phase the data are only suggestive. It seems clearly shown that the incidence of decay caused by organisms was lower in roots which were well-supplied with phosphorus than in phosphorus-deficient roots, and that the tissues were more resistant to the advance of organisms when invasion occurred.

Although in some cases the results indicate that other food materials which were supplied might also have improved keeping quality, these seem secondary to the phosphate effect. Under the fertility conditions at Hyde Park in the 1932 experiment, the decay of the sugar beets, as measured by the percentage of tissue decayed, did not differ significantly in plots receiving superphosphate and those receiving ammonium sulphate (adequate soil-moisture series). If the ammonium sulphate plots receiving manure are compared with those not receiving manure, it is seen that the difference is significant as measured in percentage and in weight of decayed tissue, whereas manure applications did not affect the plots fertilized with superphosphate. From this it seems reasonable to attribute the effects to phosphate relations primarily. Records as to foliage conditions indicate that the nitrogen additions were not large enough to induce a phosphate deficiency in the plants, but rather that they had a favorable influence upon yield. The records from other experiments, such as those in which a complete

fertilizer was used and those in which, in 1933, high and low superphosphate applications were used in combination with high and low nitrate treatments, indicate that there was no deleterious effect on resistance of root tissues to decay if phosphorus deficiency was avoided.

The tests with potassium sulphate were too limited to permit any conclusion to be stated except that when used at Hyde Park in 1932 it was not outstanding in its effect on keeping quality.

The effects of partially decomposed manure when used alone or in combination with inorganic fertilizers were pronounced except in the phosphate plots with adequate moisture already mentioned. Beets grown in manured soils were, irrespective of irrigation rates, decidedly superior in keeping quality. As is well known, the effects of manure application come from a combination of factors; it is believed that the effects in this experiment can be attributed to liberating phosphate and to improving water relations.

Another clear-cut response shown in the tests reported, notably the experiment at Hyde Park in 1932, is the influence of adequate water supply for the growing plants on the production of roots capable of keeping well in storage. If the plots without manure applications in this test are considered as a whole, it is seen that in every case, irrespective of treatment, the decay in the sugar beets from the plots receiving an inadequate moisture supply significantly exceeded the decay in beets grown with enough soil moisture. The test at Payson in 1932, in which a high percentage of invasion occurred, gives further evidence in this respect. It seems reasonable to conclude that the other factors influencing growth, such as organic fertilizers and soil moisture, may have operated beneficially because of their indirect effects in making phosphates available.

The results from the inoculation experiment were in complete accord with the results of the field test in that roots grown with an adequate supply of phosphates were markedly more resistant to invasion and decay by *Phoma betae* than undernourished roots.

From these experiments the conclusion is drawn that under the conditions of the experiments, sugar beets that were well nourished, especially with respect to phosphate, and adequately watered, showed less deterioration by decay when stored than did beets grown under less favorable conditions. Some indication was found in the tests where analyses of the stored roots were made that beets fertilized with phosphate showed lower losses from respiration than those not fertilized. The results obtained indicate accessory benefits to be derived from the use of manure and commercial fertilizers in growing sugar beets. Sugar beets from fields in which high yields indicate favorable conditions for growth may be expected to keep best in storage. In normal campaigns of 80 to 100 days, many thousands of tons of sugar beets are accumulated in piles and may remain in storage 40 to 50 days or even longer before being processed. With storage conditions continuing as at present, the relative resistance to deterioration shown by properly nourished roots must have an important bearing on loss reduction.

SUMMARY

Sugar-beet roots grown under conditions of adequate and inadequate phosphate supply were obtained for comparison as to keeping quality from Utah fields known to be deficient in available phosphate.

Roots from plots receiving superphosphate or a complete fertilizer high in phosphate showed less decay than roots from unfertilized plots. In these tests the roots were placed in coarse-meshed net sacks and exposed to conditions of commercial storage piles. There were indications that, aside from reduction of loss by decay, the phosphate fertilization reduced the loss of sucrose reserves due to respiration.

In an experiment in which other factors influencing growth of plants were considered, phosphate fertilization and, to some extent, fertilization with nitrogenous fertilizers improved keeping quality. Adequate moisture during the growing season was found to be favorable. Barnyard manure applied to the plots showed beneficial effects on keeping quality; this was believed to be chiefly because of its content of available phosphate, its indirect action in making soil phosphate available, and its effect on soil moisture.

Inoculations with *Phoma betae* corroborated the conclusion drawn from field tests, since sugar beets grown with an adequate phosphate supply showed significantly less decay than unfertilized roots used as controls.

In making up storage piles at the factory, if care is taken to store sugar-beet roots that have been grown under the conditions found conducive to good keeping quality, losses from decay and, in some measure, respiration losses should be lessened.

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THE PRODUCTION OF MILK OF ABNORMAL COMPOSITION BY ANIMALS FREE FROM UDDER STREPTOCOCCI [1]

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INTRODUCTION

Chronic mastitis of the dairy cow has attracted much attention during the years of the present decade. Numerous papers have presented the findings of many research workers; varied suggestions, based on these findings, have been made to producers and to distributors. Regulatory agencies have been influenced. Indeed all agencies having to do with milk have become aware of chronic mastitis in the dairy cow.

The prevailing opinion is that:

1. Approximately 90 percent of the cases of chronic mastitis are due to *Streptococcus agalactiae;* the remainder to other bacteria, such as other kinds of streptococci, staphylococci, micrococci, and varieties of *Escherichia coli.*
2. *Streptococcus agalactiae* grows only in the udder.
3. It is carried from infected to noninfected udders.
4. The most frequent vehicle is the milker, human or mechanical.
5. The infected quarters do not produce a proportionate amount of milk.
6. The disease shortens the milk-production period.
7. The milk produced by infected quarters is changed in composition.
8. The organism is not pathogenic for man.

The acceptance of these statements leads the milk producer to the conclusion that the disease is of economic significance to him; the milk consumer to the conclusion that infected animals should be removed from the herd because the milk produced by them is abnormal. The public health official recognizes in acute mastitis an implied danger since it is known that *Streptococcus epidemicus* of the human type may cause a severe disturbance in the udder of the cow as well as septic sore throat in man. Milk free from all streptococci cannot cause septic sore throat; hence the consumer's desire to have his supply free from these organisms. Thus it would seem that it is to the interest of all persons concerned with milk to have cows with mastitis eliminated from the herds producing milk for the fluid-milk market insofar as this is reasonably possible. It is not sufficient to consider the qualitative aspects alone; the quantitative must also be considered, although the unity of interest may be disturbed by such a consideration of the problem.

Chronic mastitis is a bacteriological problem. Its diagnosis may be made in a direct manner by proving the presence of the causal organism, or indirectly by proving the milk to be abnormal in some respect. The direct manner of proof is expensive and involved; the

[1] Received for publication June 30, 1936; issued Feb. 1, 1937.

indirect manner is cheap, easy, and adapted to use in the field. Practically all regulatory work is accomplished through the use of indirect methods rather than direct. The physical examination of the udder to detect changes in the tissue, due to the growth of organisms therein, is also widely used.

The purpose of this paper is to present certain findings and to consider their implication in relation to the problem of chronic mastitis. Normal animals and normal milk represent the ideal. If all cows are free from *Streptococcus agalactiae*, will the ideal of a normal milk be approached, or if all cows producing abnormal milk are removed, will only cows harboring this organism be removed? In other words, how safe are the foundations of our present ideas?

The standards which are commonly accepted in regard to normal milk are:

1. That it is free from visible particles.
2. That it contains less than 0.14 percent of chlorine in the form of chlorides (chlorine value).
3. That it yields less than 15 percent of its volume of 0_2 when mixed with hydrogen peroxide (catalase value).
4. That it has a true acidity represented by a pH of 6.5–6.8.

EXPERIMENTAL PROCEDURE

THE HERD

The animals were 1 year old at the time they were assembled. They were free from tuberculosis and Bang's disease and were placed on a farm on which there had been no cattle for 8 months. The stables had been cleaned and disinfected immediately after the removal of the previous herd. It was thought that both environment and animals would be free from *Streptococcus agalactiae*, and it was hoped that they would so remain during the first lactation period at least. The plan was to make no additions, other than a sire, to the herd, which was primarily for the study of pasture management. The animals had no contact with other bovines. It was therefore an isolated herd of Holstein-Friesian heifers that came to their first lactation period about 1 year after the animals were assembled.

THE MILK SAMPLES

The "routine" milk samples consisted of the first 30 cubic centimeters drawn from each quarter into a sterile bottle with no other precautions as to cleaning the udder and teats than were used in the usual procedure of milking. It was thought that additional precautions to prevent contamination of the milks from external sources were not necessary, a conclusion that has been justified by experience, since there is no reason to believe that such contamination has confused the picture. The foremilk samples are referred to in the discussion as "routine" samples. In the case of some of the animals they were supplemented by samples representing the remainder of the milk of each quarter for the particular milking. Such samples are referred to in the text as "special" samples and in table 5 as "whole milk."

EXAMINATION OF THE MILK

During the first 8 months the samples were collected at the evening milking, were refrigerated overnight, and the next day were examined, before being mixed, for any physical abnormalities such as flocs,

wateriness, and sediment. Slides, to be stained according to the Newman method, were made from the cream layer in which bacteria and leucocytes would be concentrated. During the last 8 months the samples were collected in the morning and studied the same day. Microscopic examination was not continued during this period, since it supplied little or nothing of value beyond that furnished by the other tests.

During the early part of the study the milks were placed in two groups: One consisted of the milks containing less than 0.15 percent of chlorine; the other, of those showing a greater percent of chlorine. Later the chlorine content was quantitatively determined by titration with a solution of silver nitrate, dichlorofluorescein [2] being used as an indicator. Five cubic centimeters of milk was diluted with 20 cubic centimeters of distilled water, and the silver nitrate solution added until a faint pink color developed. Somewhat more consistent results can be obtained with dichlorofluorescein than with the chromate because the end point is more definite. The wet ashing method is believed to be the most accurate one for determining the chlorine content of milk. It yields lower results than are yielded by the titration method in which potassium chromate is the indicator. The results yielded by dichlorofluorescein fall between those supplied by the other two and, therefore, probably represent a closer approximation to the truth than those yielded by the chromate method.

The catalase value represents O_2 set free from a mixture of 2 parts of milk and 1 part of a 1.5-percent H_2O_2 solution in 24 hours at 20° C., expressed as percent of the milk. The catalase content is an indirect measure of the content of the milk in leucocytes and tissue cells, either in the form of recognizable cells or of cell debris. Fresh bovine blood serum, free from cells, contains no catalase. The bacteria in the milk as drawn were probably always too few to supply any appreciable amount of catalase.

The pH was determined by the colorimetric method suggested by Brown.[3] The total acidity was measured by titrating with N/10 NaOH solution, with phenolphthalein as the indicator.

The milks were cultured by spreading about one three-hundredth of a cubic centimeter of cream, or of the mixed sample, over the surface of a slope of glucose agar, free from water, and by placing an equal amount in a tube of litmus milk. The tubes of agar and of milk were incubated at 37° C. and examined after 24, 48, 72, and 96 hours for relative numbers of colonies, for colonies of streptococci, and for the characteristic changes produced in litmus milk by *Streptococcus agalactiae*, namely, an acid curd, and no reduction of the litmus, or reduction confined to the lower half of the column of milk. Suspicious colonies on the agar slopes and suspicious tubes of litmus milk were examined microscopically, and, if the circumstances indicated, pure cultures were studied in detail. The special samples were subjected to the routine tests and to more detailed cultural examination, such as plating on blood agar, on glucose agar made with veal infusion or with beef extract, and on the same agar containing enough sterile milk to give an evident opacity to the layer of medium in a Petri-dish culture. Some cultures were incubated under anaerobic condi-

[2] KOLTHOFF, I. M., LAUER, W. M., and SUNDE, C. J. THE USE OF DICHLOROFLUORESCEIN AS AN ADSORPTION INDICATOR FOR THE ARGENTOMETRIC TITRATION OF CHLORIDES. Jour. Amer. Chem. Soc. 51: 3273-3277. 1929.

[3] BROWN, J. H. THE COLORIMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF SMALL AMOUNTS OF FLUID. Jour. Laboratory and Clin. Med. 9: 239-244, illus. 1924.

tions. In fact numerous efforts were made to find an explanation for the conditions noted.

THE CRITERIA

The criteria used in classifying the milk as abnormal were a pH of 6.8 or above, a catalase value of 50 or above, and a chlorine content of 0.15 percent or above. These criteria may be questioned. Some may insist that a catalase value of 50 is too high. The data of table 1 present a general picture of the findings. At any period of observation the milk of any animal was classed as abnormal when the product from one or more quarters conformed to at least two of the three criteria of abnormality mentioned above. The data have been compiled with consideration of the influence of the colostral period and of late lactation on the composition of the milk. It is believed that the length of the period of observation, the number of observations, and the type of samples and of the tests were such as to give a basis for valid conclusions.

TABLE 1.—*Occurrence of foremilk of abnormal composition in animals free from udder streptococci*

Animal no.	Length of the period of observation	Observations	Times milk was—		Rating of animal	Animal no.	Length of the period of observation	Observations	Times milk was—		Rating of animal
			Normal	Abnormal					Normal	Abnormal	
	Days	*Number*	*Number*	*Number*			*Days*	*Number*	*Number*	*Number*	
1	245	17	17	0	Normal.	27	156	14	12	2	Normal.
2	183	12	12	0	Do.	28	344	25	7	18	Abnormal.
3	240	15	2	13	Abnormal.	29	250	22	4	18	Do.
5	321	34	3	31	Do.	31	318	36	17	19	Do.
6	307	33	33	0	Normal.	32	309	30	25	5	Normal.
7	285	30	29	1	Do.	33	198	20	20	0	Do.
8	322	32	32	0	Do.	34	315	31	17	14	Abnormal.
10	268	18	9	9	Abnormal.	35	259	14	1	13	Do.
11	275	23	0	23	Do.	36	233	19	17	2	Normal.
13	371	29	22	7	Normal.	37	371	32	32	0	Do.
14	317	30	26	4	Do.	38	363	33	32	1	Do.
15	349	34	5	29	Abnormal.	39	320	29	21	8	Abnormal.
19	329	27	3	24	Do.	40	274	31	23	8	Do.
21	268	30	5	25	Do.	42	244	11	11	0	Normal.
24	198	20	20	0	Normal.	44	294	29	24	5	Do.
26	163	15	14	1	Do.						

THE FINDINGS

The results of the examination of the 31 cows, mentioned in table 1, and of the 3,100 samples therefrom for *Streptococcus agalactiae* were wholly negative. The abnormalities noted in the milks were due to other causes than this organism. This point will be discussed in greater detail later.

It is evident from table 1 that only 8 animals (i. e. those that yielded no abnormal samples) out of the 31 can be considered as having supplied no evidence of mastitis throughout the period of observation. Any one of the remaining animals might have been judged as producing milk unfit for direct consumption if a strict interpretation of the tests had been made. The assumption might also have been that the udder was infected with *Streptococcus agalactiae* and the removal or segregation of the animal advisable to lessen the spread of chronic mastitis in the herd.

TABLE 2.—*Condition of the herd as indicated by chlorine, catalase, and pH values of milk of each animal on different dates*

Date	Data for cow No.—																													Total	
	1	2	3	5	6	7	8	10	11	13	14	15	19	21	24	26	27	28	29	31	32	33	34	35	36	37	38	39	40	42	44	Normal	Abnormal
Apr. 1, 1935	----	----	----	+	−	−	−	----	+	+	−	−	----	+	----	----	----	----	----	−	−	----	----	----	----	−	−	−	−	----	−	*Number*	*Number*
May 1, 1935	----	----	----	+	−	−	−	----	+	+	−	+	+	+	−	----	----	−	----	+	−	−	−	----	----	−	−	−	−	----	−	12	4
June 1, 1935	−	----	----	−	−	−	−	−	+	+	−	+	+	+	−	−	−	−	−	−	−	−	−	----	----	−	−	−	−	----	−	14	7
July 1, 1935	−	−	+	+	−	−	−	−	+	−	−	+	+	+	−	−	+	+	+	+	−	−	+	----	−	−	−	−	+	----	−	22	5
Aug. 1, 1935	−	−	+	+	−	−	−	−	+	−	−	+	+	+	−	−	−	+	+	+	+	−	+	−	−	−	−	+	−	−	−	16	13
Sept. 1, 1935	−	−	+	+	−	−	−	+	+	−	−	+	+	+	−	−	−	−	+	+	+	−	+	+	−	−	−	+	+	−	−	19	12
Oct. 1, 1935	−	−	+	+	−	−	−	+	+	−	−	+	+	+	−	−	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	17	14
Nov. 1, 1935	−	−	−	+	−	−	−	−	+	−	−	+	−	+	----	+	−	+	+	+	−	−	+	+	+	−	−	+	+	−	+	15	16
Dec. 1, 1935	−	−	+	+	−	−	+	+	+	−	−	+	+	+	−	−	−	+	+	+	−	−	+	+	+	−	−	+	+	−	−	18	12
Jan. 1, 1936	−	−	−	+	----	----	−	+	+	−	+	+	+	----	----	----	----	----	+	−	+	----	+	+	+	+	−	−	----	−	−	13	13
Feb. 1, 1936	−	----	+	----	----	----	----	+	+	−	−	----	+	----	----	----	----	+	+	----	----	----	+	+	+	−	−	+	----	−	+	9	13
Mar. 1, 1936	----	----	+	----	----	----	----	+	+	−	−	----	+	----	----	----	----	+	----	----	----	----	----	+	----	−	+	----	----	−	----	5	7
Apr. 1, 1936	----	----	+	----	----	----	----	----	----	−	−	----	+	----	----	----	----	+	----	----	----	----	----	+	----	----	----	----	----	−	----	4	6
																																3	4

[1] −=milk was normal; +=was abnormal.

An examination made on any date would have indicated only a portion of the animals as abnormal. Thus, an inspection at monthly intervals throughout the year would have yielded the results presented in table 2. It is to be noted that, on the basis of the total number of examinations made, animals 1, 2, 6, 8, 24, 33, 37, and 42 in table 1 would be classed as normal since none of these supplied a sample of abnormal milk. On the basis of an examination made on the 1st of every month for 13 months, the same animals would have been classed as normal, and also no. 7 which, as is shown in table 1, yielded but one sample of abnormal milk.

If the herd had been inspected for mastitis on June 1, 1935, 5 of the then 27 milking animals would have been considered affected. An inspection as of December 1, 1935, would have revealed 26 milking animals, 13 of which would have been classed as abnormal.

It is to be noted from table 1 that some of the animals were classed as normal in spite of the occurrence of abnormal milk. Animal 13 is an example. The distribution of the abnormal samples was such as to relate the abnormality to the colostral period or to some transitory condition in a quarter. Inasmuch as the purpose of this paper is to present the record of certain of the animals about the abnormality of which there can be no question, any discussion about either the normal or the questionably normal has been omitted. There were 17 such in the total of 31 and 14 definitely abnormal animals. Detailed data are presented for seven of these. The data for the others are omitted since they would add nothing to the picture.

THE ABNORMAL ANIMALS

A table has been prepared in which the figures represent the approximate number of days from the beginning of the lactation period to the date of sampling. A plus sign means that the sample satisfied two of the three criteria previously mentioned and that the foremilk was, therefore, classed as abnormal; a minus sign that two of the three criteria were not satisfied and that the milk was considered normal. Thus, it is seen from table 3 that quarter 1 of cow 3 supplied no abnormal samples; quarter 2, 2; quarter 3, 4; while the milk from quarter 4 was normal but twice.

It is to be noted from table 3 that one quarter of cow 11 produced abnormal milk throughout the entire period of observation, 275 days, while the other quarters always produced normal milk. The detailed data of the routine samples from this animal are given in table 4. It is seen that every sample from quarter 2 was abnormal in respect to each of the criteria used in the routine examinations except the pH in the first and last samples, while every sample other than the first two taken from quarter 1 was normal or did not satisfy any two of the three criteria of abnormality. Samples of foremilk and of the remainder produced by each quarter were taken at five periods (table 5). It is to be noted that quarter 2 produced a much smaller quantity of milk than the other hindquarter, no. 3, and that the abnormality was not confined to the foremilk but was present in the entire quantity, termed "whole milk" in the table, from quarter 2, at any milking. The pH of both foremilk and the entire quantity was generally high and the titratable acid low. Quarter 2 thus differed significantly from the others as regards the composition and yield of milk.

TABLE 3.—*Normality or abnormality of the foremilk of the four quarters of the udder of the abnormal cows at various times after their lactation periods began, as indicated by the ability or inability of the sample to satisfy the three criteria mentioned in the text* [1]

Days after lactation period began	Date for indicated quarter [2] of cow no.—																													
	3				11				15				29				19				21				5				31			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
7	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−				
14	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−				
21	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	+	−				
28	−	−	+	+	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−				
35	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
44					−	+	−	−	−	+	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
51									+	+	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
56	−	−	−	+	−	+	−	−	−	+	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
63					−	+	−	−	+	+	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
70	−	−	−	+	−	+	−	−	−	+	+	−	−	+	−	−	−	−	−	+	−	+	−	−	−	+	−	−				
75									+	+	+	−					−	−	−	+	−	+	−	−	−	+	−	−				
79					−	+	−	−					−	−	−	+					−	+	−	−	−	−	−	−	−	−	−	−
85					−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−
92	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−	−
98									+	+	+	−					+	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−
104					−	+	−	−													−	+	−	−	−	−	−	−	−	−	−	−
112					−	+	−	−													−	+	−	−	−	+	−	−	−	−	−	−
115									+	+	+	−	−	−	−	+									+	−	−	−	−	−	−	−
121	−	−	−	−					+	+	+	−	−	−	−	+	+	−	−	+	−	+	−	−								
126					−	+	−	−	+	+	−	−	−	−	−	+					−	+	−	−	+	−	−	−	−	−	−	−
135	−	−	−	+					+	+	+	−					+	−	−	+	−	+	−	+	+	−	−	−	−	−	−	−
142									+	+	+	+					−	−	−	+	−	+	−	+	+	−	−	−	−	−	−	−
147	−	−	−	+					+	−	−	−									−	+	−	+	−	+	−	−	−	−	−	−
155					−	+	−	−	+	+	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	−	−				
161									+	+	+	−	−	−	−	+	−	−	−	+	−	+	−	+	−	+	−	−	−	−	+	−
167																					−	+	−	−	−	+	−	−				
173									+	+	+	−													−	−	+	−	−	−	−	−
176	−	−	−	−																												
181					−	+	−	−					−	−	−	+	−	−	−	−	+	+	−	+	−	−	+	−	−	−	−	−
188					−	+	−	−	+	+	+	−	−	−	−	+	+	−	−	+									−	−	−	−
198									+	+	+	−																	−	−	−	−
203									+	−	−	−									−	+	−	−	−	−	+	−	−	+	+	−
210	−	−	−	+	−	+	−	−									+	−	−	−												
215													−	−	−	−									−	−	+	−	−	−	+	−
224					−	+	−	−	+	+	+	−									−	+	−	−					−	−	+	−

[1] Plus signs indicate that the sample satisfied 2 of the 3 criteria for abnormality mentioned in the text, minus signs that it did not.
[2] 1 is the right forequarter; 2, the right hindquarter; 3, the left hindquarter; and 4, the left forequarter.

TABLE 3.—*Normality or abnormality of the foremilk of the four quarters of the udder of the abnormal cows at various times after their lactation periods began, as indicated by the ability or inability of the sample to satisfy the three criteria mentioned in the text*—Continued

Days after lactation period began	Date for indicated quarter of cow no.—																													
	3				11				15				29				19				21				5				31			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
237	−	−	−	+					+	−	+	−					−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−
245					−	+	−	−	+	+	+	+																	−	+	+	−
250									+	+	+	−	−	−	−	+									−	−	+	+	−	+	+	−
260																	−	−	−	−	−	+	−	−					−	+	+	−
266																					−	+	−	−	−	−	+	−	−	+	+	−
275					−	+	−	−	+	+	+	+					−	−	−	+												
282									+	+	+	+																	−	+	+	−
292																									−	−	+	−				
302									+	+	+	−					−	−	−	−					+	−	+	−	−	+	+	−
321																									−	−	+	−	−	+	+	−
328																	−	−	−	+									−	+	−	−
349									+	+	−	+																	−	+	+	−
378																													−	+	+	−

TABLE 4.—*The pH, chlorine content, and catalase value of the foremilk from the different quarters*[1] *of the udders of the definitely abnormal cows at various times after their lactation periods began*

COW NO. 11

Days after lactation period	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	pH	Cl[2]	Catalase[3]	pH	Cl[2]	Catalase[3]	pH	Cl[2]	Catalase[3]	pH	Cl[2]	Catalase[3]
		Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
4	6.6	+	113	6.4	+	175	6.4	+	34	6.5	+	22
12	6.6	0.183	75	7.0	0.332	150	6.4	0.155	7	6.6	0.172	21
19	6.4	.152	9	7.0	.230	20	6.4	.178	10	6.5	.148	7
25	6.5	.141	2	7.0	.273	172	6.5	.141	2	6.5	.158	3
32	6.6	.152	5	7.0	.312	117	6.6	.168	12	6.6	.189	9
39	6.6	.148	4	7.0	.312	169	6.6	.148	5	6.6	.155	3
45	6.6	.158	5	6.8	.269	80	6.5	.158	4	6.6	.161	4
53	6.6	.158	6	7.0	.316	167	6.6	.155	10	6.6	.165	3
60	6.6	.144	13	7.0	.276	198	6.6	.141	13	6.6	.141	14
67	6.6	.176	17	6.9	.300	250	6.6	.158	10	6.6	.155	12
74	6.6	.161	22	7.0	.327	88	6.6	.152	25	6.6	.155	9
95	6.6	.155	23	6.8	.286	243	6.6	-------	6	6.6	.152	8
109	6.6	.182	2	7.0	.320	230	6.6	.176	14	6.6	.185	8
131	6.6	.165	3	6.9	.269	220	6.6	.165	3	6.6	.172	3
160	6.6	.152	1	7.0	.248	174	6.6	.152	13	6.6	.144	2
186	6.6	.161	5	6.9	.293	257	6.6	.168	5	6.6	.185	5
215	6.6	.168	22	6.9	.258	273	6.6	.165	5	6.6	.172	4
249	6.6	.175	44	6.8	.269	254	6.6	.148	11	6.6	.161	5
279	6.6	.158	21	6.6	.248	245	6.6	.158	0	6.6	.158	16

COW NO. 15

0	6.2	−	172	6.0	−	192	6.2	−	162	6.1	−	121
7	6.3	−	97	6.3	−	74	6.2	+	79	6.2	+	80
14	6.4	+	41	6.6	−	73	6.6	−	43	6.6	+	23
21	6.5	+	47	6.6	+	28	6.5	+	28	6.5	+	15
27	6.2	−	20	6.8	+	20	6.6	+	23	6.4	−	27
34	6.6	+	35	6.8	+	20	6.6	+	16	6.4	−	23
48	6.6	+	46	6.8	+	26	6.8	+	54	6.7	+	23
53	6.6	+	24	6.8	+	15	6.8	+	21	6.6	−	9
60	6.9	+	24	6.8	+	13	6.9	+	18	6.6	−	9
68	6.8	+	26	6.8	+	11	6.8	+	17	6.7	−	9
74	6.7	+	30	6.8	+	11	6.8	+	15	6.5	−	3
81	6.9	0.221	21	6.9	0.200	10	6.9	0.217	12	6.6	−	7
88	6.7	.207	22	6.8	.221	31	6.8	.217	17	6.7	−	2
95	6.7	.197	19	6.8	.200	9	6.7	.178	9	6.5	−	30
103	6.8	.234	60	6.8	.217	17	6.8	.238	23	6.6	0.185	10
110	6.8	.197	20	6.8	.197	13	6.8	.200	10	6.6	.185	5
116	7.0	.234	53	6.9	.221	16	6.9	.221	16	6.6	.211	19
123	6.8	.227	47	6.9	.239	24	6.7	.217	20	6.6	.221	43
130	6.9	.221	44	6.8	.211	22	6.8	.217	12	6.6	.172	16
137	6.8	.207	29	6.9	.214	16	6.8	.202	15	6.8	.189	10
144	6.8	.202	22	6.7	.193	12	6.7	.207	29	6.6	.178	10
151	6.9	.207	40	6.8	.207	30	6.8	.202	33	6.6	.178	30
158	6.8	.211	70	6.8	.211	20	6.8	.211	29	6.6	.189	17
165	6.7	.193	55	6.8	.202	17	6.8	.197	9	6.7	.172	7
186	6.8	.234	67	6.8	.242	41	6.8	.231	36	6.7	.211	49
200	6.7	.234	33	6.8	.248	31	6.9	.231	27	6.6	.224	20
222	6.9	.262	72	6.9	.262	93	7.0	.276	59	6.6	.202	27
251	6.9	.282	147	7.0	.290	160	6.9	.303	215	6.6	.182	11
277	7.0	.320	230	7.0	.354	266	7.0	.330	235	6.8	.217	17
306	6.6	.279	158	6.7	.293	175	6.7	.262	88	6.6	.225	30

COW NO. 29

1	6.3	−	110	6.2	−	70	6.0	−	46	6.2	−	156
7	6.5	−	8	6.5	−	10	6.4	−	18	6.5	−	25
15	6.5	−	11	6.5	−	9	6.5	−	12	6.5	−	19
22	6.4	−	6	6.4	−	7	6.4	−	8	6.4	−	19
30	6.4	0.144	16	6.4	0.148	6	6.4	0.158	6	6.6	0.197	58
37	6.6	.165	3	6.6	.172	9	6.5	.172	13	6.6	.193	51
43	6.6	.144	4	6.5	.138	3	6.6	.152	4	6.8	.200	57
50	6.6	.148	23	6.6	.165	13	6.5	.144	20	6.9	.282	206
57	6.4	.148	6	6.4	.152	6	6.4	.155	68	6.6	.193	40

[1] See note 2, table 3.

[2] +=0.15 percent or more; −=less than 0.15 percent.

[3] O_2 set free from a mixture of 2 parts milk and 1 part of 1.5-percent H_2O_2 solution in 24 hours at 20° C., expressed as percentage of the milk.

TABLE 4.—*The pH, chlorine content, and catalase value of the foremilk from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued.

COW NO. 29—Continued

Days after lactation period	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	pH	Cl	Catalase	pH	Cl	Catalase	pH	Cl	Catalase	pH	Cl	Catalase
		Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
64	6.6	.158	7	6.6	.165	11	6.6	.172	5	6.8	.234	49
71	6.6	.148	2	7.0	.334	223	6.6	.138	6	6.6	.155	3
78	6.8	.141	32	6.6	.148	12	6.6	.148	17	7.2	.338	260
85	6.6	.148	26	6.6	.158	23	6.6	.148	15	6.6	.207	253
92	6.6	.134	14	6.6	.138	9	6.6	.138	10	6.6	.178	68
113	6.6	.148	11	6.6	.144	9	6.6	.144	85	6.6	.239	117
127	6.6	.165	4	6.6	.172	6	6.6	.161	6	7.0	.327	244
149	6.6	.161	10	6.6	.161	5	6.6	.152	3	6.8	.245	104
178	6.6	.138	8	6.6	.138	10	6.6	.148	10	6.9	.269	259

COW NO. 19

0	6.3	—	147	6.4	—	127	6.2	—	30	6.6	+	160
6	6.4	—	147	6.4	—	122	6.4	—	53	6.7	+	122
12	6.5	—	37	6.5	—	29	6.5	—	8	6.6	0.200	182
20	6.4	—	28	6.6	—	20	6.4	—	9	6.6	.207	204
27	6.6	—	8	6.6	—	19	6.5	—	8	7.0	.321	260
35	6.7	0.172	29	6.6	0.176	21	6.6	0.152	19	7.0	.392	265
42	6.6	.148	12	6.6	.148	12	6.4	.127	8	7.0	.330	198
48	6.6	.127	7	6.6	.141	11	6.4	.124	5	6.8	.245	195
55	6.6	.178	14	6.6	.168	15	6.6	.185	9	6.8	.258	190
62	6.5	.134	8	6.4	.131	9	6.4	.124	4	6.7	.189	180
69	6.8	.134	11	6.6	.144	5	6.6	.127	7	6.7	.193	81
76	6.6	.127	9	6.6	.124	10	6.6	.117	4	6.8	.221	152
83	6.6	.131	21	6.6	.124	27	6.4	.114	21	6.8	.211	260
90	6.7	.165	37	6.6	.155	18	6.6	.127	19	6.7	.234	59
97	6.9	.178	27	6.8	.148	26	6.8	.138	9	7.0	.310	300
118	6.8	.189	54	6.6	.168	23	6.6	.138	17	7.0	.372	282
132	6.8	.193	35	6.7	.168	18	6.6	.148	17	6.8	.234	232
154	6.6	.158	17	6.6	.144	14	6.6	.148	8	6.8	.211	161
183	6.6	.141	21	6.6	.138	8	6.6	.131	7	6.6	.152	145
209	6.9	.276	79	6.6	.172	17	6.6	.168	12	6.6	.158	10
238	6.6	.175	42	6.6	.148	45	6.6	.141	8	6.6	.172	168
272	6.6	.161	29	6.6	.158	6	6.6	.138	7	6.6	.155	104
302	6.6	.152	21	6.6	.124	3	6.6	.121	3	6.6	.141	139
328	6.6	.172	27	6.6	.144	4	6.6	.127	19	6.7	.161	210

COW NO. 21

0	6.1	+	232	6.2	+	214	6.3	+	162	6.3	—	243
7	6.3	—	56	6.4	+	162	6.4	—	97	6.3	—	44
13	6.5	—	64	6.5	—	212	6.4	—	105	6.4	—	23
20	6.6	—	19	6.5	—	24	6.4	—	31	6.4	—	16
29	6.8	—	31	6.6	+	29	6.6	—	15	6.6	—	12
34	6.6	—	75	6.7	+	69	6.7	—	53	6.7	—	64
41	6.6	—	6	6.8	+	51	6.8	—	16	6.6	—	4
49	6.5	—	4	6.6	—	79	6.6	—	5	6.6	—	4
55	6.6	+	23	7.0	+	197	6.6	+	25	6.5	—	17
61	6.6	—	4	7.0	0.313	183	6.6	—	11	6.5	—	6
69	6.7	0.165	15	7.0	.327	293	6.8	0.182	14	6.6	—	10
76	6.5	—	5	6.6	.165	222	6.6	—	23	6.5	—	5
84	6.7	.165	10	7.0	.348	226	6.6	.165	15	6.6	0.134	16
91	6.7	.161	19	7.0	.343	220	6.8	.211	33	6.6	.176	30
97	6.7	.158	13	7.0	.330	213	6.7	.197	22	6.6	.161	20
104	6.6	.178	17	7.0	.351	185	6.8	.197	26	6.7	.227	61
111	6.6	.165	24	7.0	.310	210	6.6	.152	16	6.6	.131	11
118	6.6	.155	10	6.8	.328	205	6.7	.185	18	6.6	.165	12
125	6.7	.172	23	6.9	.341	216	6.6	.178	29	6.7	.172	29
132	6.7	.161	12	6.9	.316	260	6.6	.176	26	6.8	.197	36
139	6.6	.158	26	7.0	.293	287	6.7	.178	28	6.8	.202	53
146	6.8	.144	13	7.0	.307	276	6.8	.165	21	6.7	.207	120
167	6.7	.165	20	6.9	.276	245	6.7	.178	37	6.7	.182	44
181	6.8	.178	31	7.0	.316	282	6.7	.211	46	6.7	.211	69
203	6.7	.172	11	7.0	.320	260	6.6	.200	25	6.7	.202	36
234	6.6	.158	17	6.8	.269	150	6.6	.138	9	6.6	.144	13
260	6.6	.148	2	6.9	.300	194	6.6	.168	8	6.7	.207	26

TABLE 4.—*The pH, chlorine content, and catalase value of the foremilk from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued.

COW NO. 5

Days after lactation period	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	pH	Cl	Catalase	pH	Cl	Catalase	pH	Cl	Catalase	pH	Cl	Catalase
		Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
4	6.3	—	35	6.2	+	190	6.6	—	65	6.2	—	30
11	6.4	—	17	7.0	+	197	6.5	—	43	6.4	—	13
17	6.6	—	26	7.0	+	183	6.6	—	66	6.7	—	27
24	6.7	—	32	7.1	+	142	6.8	+	56	6.6	—	14
31	6.6	—	15	7.1	+	157	6.4	—	10	6.0	—	8
38	6.5	—	7	7.0	+	170	6.6	—	23	6.4	—	10
45	6.5	—	20	7.0	+	131	6.6	—	31	6.6	—	16
51	6.6	—	31	7.0	+	171	6.6	—	51	6.5	—	23
58	6.6	—	13	6.8	+	58	6.7	—	34	6.6	—	11
67	6.6	—	8	6.8	+	29	6.6	—	14	6.6	—	8
72	6.5	—	68	6.6	—	33	6.4	+	56	6.5	—	54
79	6.6	—	11	6.8	+	29	6.8	+	28	6.5	—	14
87	6.4	—	9	6.5	—	21	6.6	+	31	6.4	—	5
93	6.5	—	21	6.6	—	22	6.6	+	64	6.4	—	9
99	6.6	0.197	14	6.7	0.200	30	6.7	0.207	34	6.5	—	11
107	6.6	—	13	6.6	—	12	6.6	—	33	6.6	—	17
114	6.7	.193	25	6.7	.165	15	6.8	.176	43	6.6	—	5
122	6.8	.248	64	6.6	.197	24	6.6	.224	75	6.5	0.185	46
129	6.9	.252	100	6.7	.197	19	6.8	.231	39	6.6	.175	25
135	6.8		36	6.6		8	6.9		66	6.6		10
142	6.8	.217	44	6.9	.182	23	6.7	.255	117	6.6	.178	17
149	6.7	.182	27	6.7	.172	31	6.8	.258	111	6.6	.158	25
156	6.7	.165	30	6.6	.168	9	6.6	.221	37	6.5	.172	10
163	6.6	.200	19	6.6	.178	24	6.8	.239	74	6.6	.207	16
170	6.8	.189	28	6.6	.165	23	6.9	.234	87	6.6	.189	33
177	6.6	.200	35	6.6	.168	28	6.7	.189	176	6.6	.134	52
184	6.6	.178	23	6.7	.161	15	6.8	.193	90	6.6	.165	30
205	6.6	.172	17	6.7	.168	46	6.8	.207	60	6.6	.168	21
219	6.6	.197	27	6.6	.176	19	6.8	.253	111	6.6	.202	33
240	6.6	.185	20	6.7	.176	22	6.9	.282	76	6.6	.176	21
270	6.7	.185	20	6.6	.185	11	6.8	.296	96	6.6	.172	14
296	6.6	.185	20	6.6	.207	26	6.8	.282	155	6.6	.172	18
325	6.6	.189	25	6.7	.197	20	6.9	.276	57	6.6	.182	40

COW NO. 31

79	6.6	—	16	6.6	—	28	6.6	—	41	6.6	—	16
86	6.6	—	3	6.5	—	6	6.5	—	6	6.5	—	5
94	6.7	—	21	6.8	—	46	6.6	—	95	6.7	—	25
101	6.6	—	3	6.7	—	21	6.6	—	13	6.6	—	0
108	6.4	—	0	6.6	—	21	6.6	—	14	6.5	—	5
115	6.6	—	0	6.6	—	7	6.7	—	18	6.6	—	0
123	6.6	—	15	6.5	—	6	6.6	—	7	6.5	—	34
130	6.6	—	9	6.6	—	6	6.6	—	22	6.6	—	0
137	6.5	—	0	6.6	—	8	6.6	—	16	6.7	—	33
143	6.6	—	0	6.6	—	4	6.6	—	10	6.5	—	4
150	6.6	—	6	6.6	—	6	6.7	—	23	6.6	—	31
159	6.6	—	2	6.6	—	6	6.6	+	22	6.6	—	0
164	6.7	—	26	6.6	—	34	6.8	—	88	6.6	—	38
171	6.6	—	7	6.6	—	7	6.8	+	65	6.6	—	9
179	6.5	—	4	6.5	—	3	6.6	—	24	6.5	—	4
185	6.6	—	2	6.6	—	11	6.6	—	37	6.6	—	6
191	6.6	—	14	6.6	—	10	6.7	0.172	46	6.6	—	19
199	6.5	—	9	6.5	—	7	6.6	.182	35	6.5	—	3
206	6.4	—	3	6.6	—	3	6.7	.185	38	6.6	—	6
214	6.6	0.165	17	6.8	0.234	94	6.8	.202	85	6.6	0.158	30
221	6.6	.141	15	6.7	.168	17	6.8	.193	40	6.6	.144	19
227	6.6	.152	9	6.7	.178	16	6.8	.202	65	6.5	.155	7
234	6.6	.161	20	6.6	.207	45	6.7	.221	68	6.6	.172	16
241	6.6	.134	7	6.7	.182	39	6.9	.234	90	6.6	.158	7
248	6.6	.141	9	6.6	.207	49	6.7	.224	145	6.6	.148	4
255	6.6	.127	0	6.8	.197	96	6.8	.202	88	6.4	.144	11
262	6.6	.141	52	6.6	.214	92	6.6	.239	32	6.6	.141	31
270	6.6	.141	23	6.6	.189	100	6.7	.224	50	6.6	.144	18
277	6.7	.148	6	6.9	.224	92	6.9	.211	164	6.7	.155	14
298	6.7	.158	29	6.8	.189	75	6.9	.224	208	6.6	.158	20
312	6.6	.148	12	6.8	.202	66	6.8	.224	215+	6.6	.161	14
334	6.6	.158	17	6.9	.217	78	6.8	.252	114	6.7	.172	16
353	6.6	.155	12	6.6	.217	82	6.7	.248	123	6.6	.189	21
379	6.6	.224	36	6.6	.227	60	6.6	.269	85	6.6	.265	45

All routine samples from animal 11 were cultured on various media and the cultures kept at 30° or at 37° C. None of the cultures made on agar slopes or in milk showed any significant result, either from the quantitative or qualitative point of view. Quarter 2 did not yield results materially different from those yielded by the other quarters. The quantitative results obtained with blood agar plates incubated 48 hours at 37° for both the fore milk and the whole milk are presented in table 5. It is to be noted that the foremilk was usually lower in bacteria than the remainder of the milk drawn from any quarter at any milking. It is also to be noted that the whole milk from quarter 2 was consistently higher in bacteria than that from the normal quarters. The plates seeded with the milk of the different quarters presented much the same appearance as to types of colonies. One gained the impression that the higher numbers in quarter 2 indicated an increase in the normal flora rather than an organism not usually present in the udder of cows. The milk from quarter 2 was never abnormal in appearance except that the fat-free milk had a watery appearance. Flakes were not noted. The quarter was not sore at any time and appeared normal when examined in a cursory manner, but a physical examination by a veterinarian skilled in such examinations revealed a fibrotic condition. The milk flow of the animal was well maintained. The maximum per week was during the first month after freshening—about 275 pounds. Eight months later about 150 pounds per week were produced.

TABLE 5.—*Yield, pH, acidity, chlorine content, catalase value, and bacterial count, of both the foremilk and the remainder of the milk (whole milk) from the different quarters*[1] *of the udders of the definitely abnormal cows at various times after their lactation periods began*

COW NO. 11, FRESHENED JUNE 7, 1935

Date	Quarter	Yield		Catalase		pH		Acidity		Chlorine		Bacteria per cubic centimeter in—	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Lb.	*Percent*[2]	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Hundreds*	*Hundreds*
Aug. 29	1	4.1	27	7	14	6.6	6.6	-------	0.157	0.144	0.152	1	16
	2	1.0	7	242	236	6.8	6.6	-------	.067	.231	.248	1	35
	3	6.0	39	17	68	6.6	6.6	-------	.139	.138	.148	1	13
	4	4.2	27	0	16	6.6	6.6	-------	.139	-------	.148	1	12
Total		15.3											
Sept. 5	1	4.5	28	2	9	6.6	6.6	-------	.157	.155	.141	7	9
	2	1.0	6	164	156	6.9	6.9	-------	.067	.258	.255	2	40
	3	6.1	38	9	7	6.6	6.6	-------	.057	.148	.138	1	5
	4	4.4	28	4	9	6.6	6.6	-------	.057	.148	.148	1	5
Total		16.0											
Oct. 2	1	3.7	37	19	10	6.6	6.6	0.121	.156	.172	.168	1	3
	2	.3	3	273	111	6.8	6.8	.040	.045	.312	.242	1	16
	3	3.6	37	14	8	6.6	6.6	.135	.156	.172	.158	1	11
	4	2.3	23	9	15	6.6	6.6	.144	.156	.172	.161	1	11
Total		9.9											
Dec. 17	1	5.5	4	8	9	6.6	6.6	.135	.144	.172	.155	40	9
	2	.07	1	214	252+	6.8	7.0	.045	.063	.303	.258	29	74
	3	4.0	29	13	12	6.6	6.6	.144	.144	.155	.152	2	20
	4	4.0	29	35	30	6.6	6.6	.139	.135	.158	.168	14	72
Total		13.57											
Jan. 22	1	3.8	29	6	11	6.6	6.6	.135	.135	.168	.161	88	92
	2	.1	1	286	145	6.8	6.8	-------	.126	-------	.172	200	106
	3	5.3	40	11	6	6.6	6.6	.144	.153	.155	.158	76	3
	4	4.0	30	19	9	6.6	6.6	.144	.144	.168	.168	4	84
Total		13.2											

[1] See note 2, table 3.
[2] Of total.

TABLE 5.—*Yield, pH, acidity, chlorine content, catalase value, and bacterial count, of both the foremilk and the remainder of the milk (whole milk) from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued

COW NO. 15, FRESHENED MARCH 13, 1935

Date	Quarter	Yield		Catalase		pH		Acidity		Chlorine		Bacteria per cubic centimeter in—	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Lb.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Hundreds*	*Hundreds*
Sept. 18	1	1.8	19	40	30	6.9	6.8	0.072	0.096	0.231	0.202	110	172
	2	3.1	33	54	32	6.8	6.8	.072	.090	.239	.214	1	90
	3	2.4	26	28	19	6.9	6.8	.063	.099	.239	.202	57	34
	4	2.1	22	20	13	6.7	6.6	.081	.117	.214	.185	1	568
Total		9.4											
Oct. 24	1	1.8	19	105	93	6.7	6.6	.081	.117	.245	.197	115	20
	2	2.8	29	37	89	6.7	6.7	-------	.099	.217	.214	2	28
	3	2.5	26	50	122	6.8	6.7	-------	.108	.207	.227	2	48
	4	2.5	26	17	17	6.7	6.6	.135	.126	.172	.178	15	14
Total		9.6											
Nov. 8	1	1.4	17	243	170	7.0	6.8	.063	.117	.282	.200	111	73
	2	2.1	25	187	227	7.0	6.8	-------	.099	.290	.224	29	61
	3	2.5	29	208	199	7.0	6.9	.054	.081	.310	.238	7	92
	4	2.5	29	18	81	6.8	6.6	.135	.135	.172	.168	56	23
Total		8.5											
Dec. 19	1	1.0	14	224	192	6.9	6.7	.063	.103	.310	.224	216	26
	2	1.5	21	260	282	7.0	6.8	.036	.072	.344	.310	3	20
	3	2.2	30	240	223	7.0	7.0	.045	.049	.324	.282	12	24
	4	2.6	35	50	44	6.6	6.6	.126	.126	.182	.168	3	39
Total		7.3											
Jan. 23	1	.3	13	117	43	6.8	6.8	.126	.144	.234	.182	228	260
	2	.1	4	132	139	6.8	6.8	.126	-------	.224	.217	34	400
	3	.7	29	47	58	6.6	6.7	.135	.135	.197	.203	35	146
	4	1.3	54	14	29	6.8	6.8	.153	.144	.158	.182	11	200
Total		2.4											

COW NO. 29, FRESHENED MAY 20, 1935

Sept. 17	1	2.1	18	10	14	6.5	6.5	0.126	0.162	0.155	0.144	1	5
	2	3.7	32	9	19	6.6	6.5	.144	.166	.155	.147	42	5
	3	4.1	35	10	12	6.6	6.5	.153	.171	.155	.147	2	33
	4	1.8	15	199	233	7.0	6.8	.058	.112	.303	.214	35	21
Total		11.7											
Oct. 23	1	2.3	18	32	50	6.7	6.6	.135	.162	.134	.134	1	5
	2	4.2	34	26	11	6.6	6.6	-------	.153	.138	.131	1	4
	3	4.5	36	27	39	6.6	6.6	.135	.162	.141	.134	1	7
	4	1.5	12	267	313	7.0	6.9	.063	.090	.282	.224	5	13
Total		12.5											
Nov. 20	1	1.1	17	25	50	6.6	6.6	-------	.157	.161	.161	2	40
	2	2.2	35	24	40	6.5	6.6	.162	.153	.155	.161	1	2
	3	2.4	38	23	41	6.5	6.6	.171	.162	.152	.155	33	6
	4	.6	10	77	143	6.7	6.6	.153	.139	.197	.178	20	32
Total		6.3											
Dec. 12	1	2.1	20	20	27	6.6	6.6	.162	.162	.155	.152	4	69
	2	3.4	32	14	20	6.6	6.6	.162	.162	.158	.155	29	34
	3	4.0	38	14	11	6.6	6.6	.189	.171	.144	.148	4	3
	4	1.0	10	39	90	6.6	6.6	.175	.149	.148	.189	63	96
Total		10.5											

TABLE 5.—*Yield, pH, acidity, chlorine content, catalase value, and bacterial count, of both the foremilk and the remainder of the milk (whole milk) from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued

COW NO. 19, FRESHENED MAY 15, 1935

Date	Quarter	Yield		Catalase		pH		Acidity		Chlorine		Bacteria per cubic centimeter in—	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Lb.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Hundreds*	*Hundreds*
Oct. 3	1	3.3	23	22	34	6.6	6.6	0.162	0.144	0.144	0.158	------	------
	2	4.4	31	10	21	6.6	6.6	.171	.153	.138	.148	------	------
	3	5.6	39	17	12	6.6	6.6	.178	.162	.134	.134	------	------
	4	1.0	7	107	246	6.7	6.7	.144	.144	.161	.165	------	------
Total		14.3											
Oct. 22	1	2.6	21	33	34	6.6	6.6	------	.144	.185	.148	50	21
	2	4.2	34	34	17	6.6	6.6	------	.144	.155	.131	40	17
	3	4.6	38	7	11	6.6	6.6	.135	.162	.144	.131	1	11
	4	.8	7	183	232	6.9	6.8	.126	.135	.176	.168	734	68
Total		12.2											
Nov. 19	1	3.2	22	40	44	6.9	6.6	.099	.162	.197	.141	135	21
	2	4.6	32	23	12	6.7	6.6	.108	.162	.172	.138	10	18
	3	5.7	39	5	10	6.6	6.6	.144	.171	.141	.124	2	24
	4	1.0	7	159	232	6.7	6.7	.153	.144	.178	.134	464	176
Total		14.5											
Jan. 30	1	2.7	20	13	63	6.7	6.7	.144	.144	.182	.158	58	28
	2	4.0	30	22	14	6.6	6.6	------	.162	.155	.148	5	36
	3	5.7	43	10	13	6.6	6.6	.162	.180	.138	.138	15	33
	4	.9	7	114	180	6.6	6.7	.182	.162	.144	.155	90	108
Total		13.3											

COW NO. 21, FRESHENED MAR. 29, 1935

Aug. 28	1	3.7	28	21	27	6.6	6.4	------	0.157	0.158	0.121	2	60
	2	2.3	18	255	240	6.8	6.6	------	.126	.316	.161	244	140
	3	3.5	27	43	50	6.6	6.6	------	.144	.161	.134	3	3
	4	3.6	27	55	70	6.6	6.6	------	.162	.134	.127	232	28
Total		13.1											
Sept. 3	1	3.4	29	28	14	6.7	6.6	------	.162	.158	.121	1	2
	2	2.0	18	171+	178	6.8	6.7	------	.121	.276	.172	238	12
	3	2.8	24	27	29	6.6	6.6	------	.157	.176	.127	8	10
	4	3.4	29	60	24	6.6	6.6	------	.153	.172	.121	94	34
Total		11.6											
Nov. 8	1	1.4	17	54	13	6.6	6.6	0.130	.153	.155	.131	5	44
	2	2.6	31	174	106	6.7	6.6	.081	.126	.217	.148	080	43
	3	2.0	24	34	6	6.6	6.6	.126	.148	.165	.127	2	14
	4	2.4	28	17	63	6.6	6.6	.139	.139	.144	.138	130	14
Total		8.4											
Dec. 20	1	2.2	30	43	55	6.6	6.6	.139	.144	.172	.138	12	19
	2	1.2	16	252	230	6.8	6.6	.063	.126	.269	.161	992	40
	3	2.2	30	26	53	6.6	6.6	------	.153	.155	.138	6	12
	4	1.8	24	28	50	6.6	6.6	------	.135	.155	.148	72	36
Total		7.4											

TABLE 5.—*Yield, pH, acidity, chlorine content, catalase value, and bacterial count, of both the foremilk and the remainder of the milk (whole milk) from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued

COW NO. 5, FRESHENED FEB. 17, 1935

Date	Quarter	Yield		Catalase		pH		Acidity		Chlorine		Bacteria per cubic centimeter in—	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Lb.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Hundreds*	*Hundreds*
Aug. 27	1	2.0	24	49	54	6.6	6.6	-------	0.144	0.168	0.127	2	6
	2	2.0	25	37	67	6.6	6.5	-------	.148	.168	.124	1	23
	3	2.0	25	87	92	6.8	6.6	-------	.135	.207	.148	42	88
	4	2.1	26	60	62	6.6	6.6	-------	.144	.144	.127	10	31
Total		8.1											
Sept. 3	1	2.3	24	28	15	6.8	6.7	-------	.148	.165	.127	2	12
	2	2.3	24	26	10	6.7	6.6	-------	.157	.158	.124	20	71
	3	2.6	27	83	43	6.8	6.6	-------	.148	.207	.138	107	52
	4	2.4	25	24	15	6.6	6.6	-------	.153	.155	.124	800	35
Total		9.6											
Nov. 1	1	1.8	25	30	17	6.6	6.6	0.099	.130	.178	.155	50	21
	2	2.1	30	22	8	6.6	6.6	-------	.144	.189	.141	29	68
	3	1.2	17	230	140	6.8	6.8	.054	.103	.300	.207	164	42
	4	2.0	28	22	17	6.6	6.6	.108	.135	.176	.148	2	30
Total		7.1											
Dec. 19	1	1.2	19	44	19	6.9	6.8	.090	.135	.197	.138	63	4
	2	2.1	34	35	27	6.7	6.6	.108	.135	.197	.144	3	3
	3	1.3	21	170	167	6.9	6.8	.054	.090	.282	.193	8	35
	4	1.6	26	20	16	6.7	6.6	.108	.144	.182	.138	6	13
Total		6.2											

COW NO. 31, FRESHENED NOV. 22, 1934

Sept. 9	1	2.4	31	3	17	6.5	6.6	0.153	0.153	0.141	0.127	2	20
	2	1.9	25	26	65	6.7	6.8	.117	.144	.189	.161	70	49
	3	1.4	18	52	72	6.9	6.6	.081	.117	.221	.168	164	25
	4	2.0	26	9	40	6.6	6.6	.144	.153	.155	.138	10	5
Total		7.7											
Oct. 25	1	1.8	39	13	37	6.6	6.6	.144	.162	.172	.158	1	39
	2	1.1	24	113	100	6.8	6.7	.099	.153	.224	.172	41	22
	3	.6	13	129	100	6.8	6.7	.117	.126	.211	.200	25	57
	4	1.1	24	13	17	6.6	6.6	.162	.171	.161	.152	1	10
Total		4.6											
Dec. 20	1	0.5	26	125	62	6.6	6.6	.135	.144	.258	.161	5	14
	2	.6	32	108	76	6.6	6.6	.135	.162	.245	.155	208	56
	3	.4	21	253	144	6.8	6.6	-------	.162	.279	.152	296	144
	4	.4	21	96	54	6.6	6.6	.135	.126	.242	.189	2	28
Total		1.9											

Quarter 2 of cow 11, secreting abnormal milk, never produced over 7 percent instead of the theoretical yield of about 28 percent of the total production. The inference is that the total yield was decreased because of the abnormality of the quarter. The correctness of such an inference is uncertain since one cannot judge the degree of compensation by the other quarters. Quarter 1, supplied by the same

blood stream as no. 2, showed no sympathetic response to the condition in no. 2. The cause of the physiological abnormalities must have been resident in the quarter. It may have been a congenital defect, which may disappear by the second lactation period, as Turner [4] has shown that the secreting tissue of the caprine udder largely disappears at the end of each lactation period and new tissue is formed at the beginning of each succeeding period. It is hoped that the animal can be observed during the second lactation period.

Cow 15 presents a different picture in that during the first 3 weeks the milk of each quarter was normal. Abnormal milk was noted first in quarter 2, 2 weeks later in quarter 3, and in quarter 1, 3 weeks later than it appeared in quarter 2, while quarter 4 yielded no abnormal sample until the one hundred and forty-second day, and not again until the two hundred and forty-fifth day.

The results of the various tests on the routine samples from cow 15 are presented in table 4. It is to be noted that the chlorine content is consistently high in all quarters and that the pH in quarter 4 exceeded 6.7 but twice—on the one hundred and thirty-seventh and on the two hundred and seventy-seventh days. The catalase content in quarter 4 did not reach 50 after the colostral period. In the case of the other quarters the abnormalities were in chlorine and in pH rather than in catalase.

The detailed data of the special samples from cow 15 are presented in table 5. It is to be noted that quarter 4 maintained its yield better than the others until on January 23, the three hundred and seventeenth day, it produced 54 percent, there being a constant increase from 22 percent on the one hundred and nintieth day. The animal produced about 300 pounds per week during the first 8 weeks of the lactation period, and showed a more rapid decrease in production than did the normal animals of the herd. It seems safe to assume that the animal produced a smaller yield of milk and fat than would have been true if the condition of each quarter at the beginning of the period had been maintained. The physical examination revealed nothing of significance.

The abnormalities were not confined to the foremilk, but were present to much the same extent in the entire amount of milk produced by each quarter.

Very low total acidity was noted; for example, the whole milk sample of quarter 3 on December 19 had an acidity of 0.049 percent, a pH of 7.0, a chlorine content of 0.28 percent, and a catalase content of 223.

The cause in the case of this animal must have increased in effect about 1 month after the lactation period began. Some disturbance must have been present from the beginning, as is evidenced by the high chlorine content constantly exceeding 0.15 percent.

The numbers of bacteria bore little relation to the extent of chemical change. This is evident in the case of the samples of December 19 which were markedly abnormal, while the bacterial content was lower than at the other periods. As with cow 11, the abnormality in bacteria seemed to be confined to an increase in the normal udder types rather than to the invasion by a new form.

Cow 29 presents a picture of an abnormal condition in the fourth quarter beginning about 1 month after freshening, while the other

[4] TURNER, C. W., and REINEKE, E. P. A STUDY OF THE INVOLUTION OF THE MAMMARY GLAND OF THE GOAT. Mo. Agri. Ex. Sta. Research Bull. 235, 23 pp., illus. 1936.

three quarters were normal with one exception, 2 on the seventieth day. The general picture is presented in table 4 and the character of the special samples in table 5. The abnormalities in the case of quarter 4 are chiefly in chlorine and in catalase, the pH being less consistently abnormal. The quarter apparently improved in condition, as is shown by the sample drawn therefrom on December 12, which was more nearly normal than any of the three special samples previously examined. The data gathered from the various types of cultures made from the special samples indicated neither such numbers nor such kinds of bacteria as to prove that they were the cause of the disturbance. Quarter 4 usually showed more bacteria than the others, table 5.

Cow 19 is comparable to cow 11 in that the samples yielded by quarter 4 were quite consistently abnormal (table 3). Quarter 1 yielded abnormal samples on the ninety-eighth, one hundred and twenty-first, one hundred and thirty-fifth, one hundred and eighty-eighth, and two hundred and tenth days. Other data on this cow are recorded in table 4.

It is to be noted from table 5 that quarter 4 of cow 19 produced only a small percentage of the milk, and the percentage did not change during the 4 months covered by the data of the special samples. The foremilk of quarter 4 was no more abnormal than the entire quantity of milk drawn from the quarter at any period of observation, a condition differing from that noted in some other animals.

The bacterial content of quarter 4 was high as revealed by the agar slopes inoculated from the routine samples, during the entire period of lactation except the first 2 months. These data were confirmed by the examination of the special samples as are presented in table 5.

Cow 21 was judged as normal for the first 4 weeks of the lactation period; subsequent thereto, quarter 2 was abnormal, as is shown in table 3. Evidences of abnormality were present early in the period, as is shown by the high chlorine content (table 4). Four special samples were taken in the last 4 months of the lactation period (table 5). Quarter 2 produced less than its normal part of the milk; however, its yield did not decline more rapidly than that of the other quarters. The catalase content of the foremilk and of the remainder of the milk from quarter 2 was much the same, while as regards acidity and chlorine the foremilk was more abnormal than the remainder. Such irregularities in abnormalities cannot be explained. The bacterial content of the routine samples was relatively high. This was confirmed by the results of the plate cultures made from the special samples.

The milk from quarters 2 and 4 did not differ significantly in numbers of bacteria but did differ in composition. The cultures of the milk of quarters 2 and 4 showed sufficient differences to attract attention. The plates seeded with the milk of quarter 2 showed many yellow colonies, while those from quarter 4 were almost completely nonchromogenic. This difference persisted in a marked degree during a number of months and to the end of the lactation period. The varying action of the two types of organisms on red blood cells and on casein showed that they were actually different, not simply different in pigment production. Whether the pigment-forming organism is the cause of the abnormal secretion or has any relation to it cannot be stated.

Cow 5 yielded from quarter 2 only abnormal samples until the samples taken on the seventieth day of lactation; thereafter this quarter yielded but two abnormal samples on the seventy-fifth and one hundred and forty-second days. Quarter 3 was quite consistently abnormal (table 4).

The detailed data of the samples are presented in tables 4 and 5.

Cow 31 produced but one abnormal sample during the first 198 days of the lactation period. On the one hundred and sixty-first day, quarter 3 yielded an abnormal sample, and again on the two hundred and third day. During the remaining 175 days, the quarter yielded 1 normal sample and 12 abnormal. Quarter 2 yielded its first abnormal sample on the two hundred and third day, its second on the two hundred and forty-fifth. Beginning then for 133 days 10 abnormal samples were collected. Quarters 1 and 4 remained normal, as is shown by table 3 to the three hundred and seventy-eighth day. On the three hundred and ninety-third day, all quarters yielded abnormal milk (table 5, samples of Dec. 20).

The detailed data of the 36 routine samples taken from each quarter of cow 31 are presented in table 4, from which it is to be noted that the abnormality in the milk from quarters 2 and 3 is marked by a high chlorine and catalase more than by an increase in pH.

The special samples were not collected until the eleventh month of the lactation period. The difference between quarters 1 and 4 as compared with 2 and 3 is evident in the data recorded in table 5 relating to production, catalase, acidity, pH, and chlorine. The bacterial data indicate a higher number of bacteria in quarters 2 and 3 than in 1 and 4 during the period in which the special samples were taken. The increase seems to be in the normal flora rather than in the presence of unusual types of bacteria.

PRODUCTION

The influence of the abnormality on the amount of milk produced cannot be ascertained with certainty from the data. Indeed, the question of the influence of chronic mastitis on milk production is one almost impossible of definite answer, for the yield of milk in one lactation period is governed by a number of factors. In any subsequent period, some change in one or more factors may occur. Such a change may not be apparent and yet may be very significant. No one can estimate the significance of compensation by the normal quarters. One must admit that statements as to the influence of chronic mastitis on milk production have little factual basis. They are surmises, and probably one may safely surmise that an animal which has a disturbed physiological function cannot produce milk with the same degree of efficiency as though the function were normal. The fact that the abnormal quarters produce less milk than the corresponding normal quarters has been presented in the detailed data of a number of the animals. The only satisfactory way of studying the influence of a chronic inflammatory process in a quarter on the milk production thereof is to determine the yield of each quarter of a number of animals at frequent intervals throughout an entire lactation period, then, to compare the condition of the quarters and the amount of milk therefrom, and especially to note whether a quarter which became abnormal at some time in the lactation

period decreased in flow more rapidly than did the normal quarters of the same animal. The milking of the abnormal animals in the herd by quarters was not begun until late in the lactation period. The data are not sufficient to be conclusive. The records of two of the animals are presented in table 6.

TABLE 6.—*Milk production of the 4 quarters*[1] *of the udders of cows 15 and 29 during periods of approximately 3 months*

Date of observation	Quarter 1		Quarter 2		Quarter 3		Quarter 4	
	Pounds	Percentage of total	Pounds	Percentage of total	Pounds	Percentage of total	Pounds	Percentage of total
COW 15								
Sept. 18	1.8	19	3.1	34	2.4	25	2.1	22
Oct. 24	1.8	18	2.8	30	2.5	26	2.5	26
Nov. 8	1.4	17	2.1	25	2.5	29	2.5	29
Dec. 19	1.0	14	1.5	20	2.2	30	2.6	36
COW 29								
Sept. 17	2.1	18	3.7	32	4.1	35	1.8	15
Oct. 23	2.3	18	4.2	34	4.5	36	1.5	12
Nov. 20	1.1	17	2.2	35	2.4	38	.6	10
Dec. 12	2.1	20	3.4	32	4.0	38	1.0	10

[1] See note 2, table 3.

Cow 15 freshened on March 13; quarters 1, 2, and 3 had been abnormal about 5 months previous to the first observation. Quarter 4, which had been normal throughout, showed a persistence in production not shown by the other quarters. When making comparisons one should keep in mind that quarters 1 and 4 are forequarters and 2 and 3 are hindquarters.

Cow 29 freshened on May 17; quarter 4 became abnormal about 4 months previous to the first observation.

Cows 5, 19, 21, and 31 showed the same tendency toward a greater decrease of production in the abnormal quarters than in the normal. The data which have been studied in other ways are too few to indicate other than probabilities. It may be said that no matter how the data are handled, the result is to indicate that the abnormal condition caused a more rapid reduction in yield than was noted in the normal quarters.

TRANSMISSION

There has been no reason to believe that the condition has spread from one quarter to another of the same animal or from one animal to another; especially does this seem a safe conclusion in view of the fact that no type of bacteria could be found to be causally related to the abnormality.

PERSISTENCE

The disturbance of function, once established, seems very persistent. Later observations will reveal whether the disturbance will again appear in the next lactation period.

In the case of cows 5, 11, 19, and 35, the condition was present very early in the period and persisted in a relatively consistent manner

throughout the period. In the case of cows 10, 15, 21, 28, 29, 31, 34, and 40, it appeared later and persisted, while in the case of cow 13 it was present early in the period and disappeared later.

SIGNIFICANCE TO PRODUCER

The significance of the disturbance to the producer cannot now be stated. If milk of abnormal composition from quarters free from udder streptococci should prove to persist throughout a number of lactation periods in the same quarter or in the same animal, or if it should prove to be a transmissible trouble, its significance would be greater than the present data indicate. It would seem unwise, considering the present paucity of knowledge regarding this condition, for a farmer to remove from his herd an animal in her first lactation period because of this type of abnormality of the mammary gland. No one is in a position to advise the farmer as to means of prevention or to suggest changes in management that might possibly correct it.

SIGNIFICANCE TO THE CONSUMER OF MILK

As has been stated, no definite agent has been found which seems to stand in causal relation to the abnormality other than possibly a growth of normal udder bacteria beyond the usual number. There is no reason to believe that the trouble has other than an aesthetic significance.

PRESENCE IN OTHER GROUPS OF CATTLE

The same abnormality has been noted in an entirely separate group of animals in the first lactation period. The incidence is much the same as in the herd discussed herein. A review of the data collected in a survey of farm herds made in 1934, using much the same methods, shows a lack of correlation between an abnormal secretion and the presence of *Streptococcus agalactiae* or other streptococci in the udder. Since but one examination of each animal was made, or at most two, the lack of correlation was considered at the time to be due to the inadequacies of the samples and of the tests used for the detection of streptococci. Thirty-nine herds, containing 711 animals, were examined, with the following result:

	Percent
Normal milk	61
Abnormal milk and streptococci	27
Abnormal milk and no streptococci	12

THE CAUSE OF THE ABNORMAL MILK

The detailed examinations as to kind and number of bacteria in the abnormal milk as compared with those in normal milk have supplied little significant information. In general, the total bacterial content of the abnormal milk is higher than that of the normal. In most instances it does not seem high enough to account for the physiological disturbance. Again, the types of bacteria impress one as being the normal udder forms. It should be remembered that nothing is known of the products produced by these bacteria, especially as they grow in the udder, and hence nothing of their pathological effect. It may be that some form of bacteria is present in the quarters supplying abnormal milk that is not present in the other quarters.

Again, the difference may be in the varying susceptibility of the tissue of different quarters to some agent of bacterial origin. It scarcely seems possible that such inequality of response to such a factor would be present without previous tissue injury. Such injury seems improbable in such a large part of a group of young animals.

It is difficult to think that similar tissues in an animal will be unlike in quality; as for example, the different quarters of the mammary gland of the cow. It is supposed that the different quarters have the same supply of nutrients and are controlled by the same supply of identical hormones, therefore, variation in quality of secretion from different quarters is difficult to conceive. Such reasoning indicates that the cause of the trouble must be an invading agent, bacterial or otherwise. On the other hand, there is plenty of evidence to show that variations in quality of milk from the quarters of an udder are present in the case of all cows. The magnitude of variation may not be great, but some variation can be detected at any milking. Physiologists consider the animal as a unit. In the present approach to chronic mastitis, the tendency is to examine the product of the different quarters, and to draw conclusions with little knowledge of the variation which may be considered normal. So far as the authors are aware, no study including the milking of a number of normal animals by quarters over a period of time, followed by a detailed study of the milks, has been carried out. Such a study is essential to any wise approach to the problem of chronic mastitis.

Many attempts have been made to produce chronic mastitis by the introduction of cultures of *Streptococcus agalactiae* into the teat duct, or by bringing them in contact with the end of the teat. Milk containing the same organism has likewise been used. The results have been disappointingly negative in spite of the commonly accepted idea that the organism slowly passes from animal to animal under natural conditions. The failure to produce the disease artificially has led many to invoke a special condition in the udder necessary for the semipermanent or permanent establishment of *Streptococcus agalactiae*. The absence of this condition at the moment the artificial infection was attempted prevents the organism from gaining a foothold, while under natural conditions, the organism obtains its opportunity by being constantly present. Because of the limitations under which the observations herein presented were made, there has been no opportunity to see whether a quarter producing an abnormal milk provides such an acceptable environment for *Streptococcus agalactiae* as to permit it to establish itself with ease and consistency.

SUMMARY

The production of milk of abnormal composition in a herd of 31 cows which had had no contact with other cattle either during or for 1 year before the period of observation was studied during the first lactation period.

Three thousand samples of foremilk from separate quarters were analyzed for chlorine, for catalase, and for pH value. Each sample was cultured on glucose agar and in milk. Additional samples of foremilk and of the entire product of each quarter from animals producing abnormal milk were examined in greater detail as to composition and bacterial content.

The abnormalities noted were such as to indicate a chronic inflammation of low intensity in the involved quarters. No streptococci were found in any sample. It is thus evident that the pathological condition was not caused by the organism, *Streptococcus agalactiae*, admittedly the most frequent cause of chronic mastitis. The abnormal condition is apparently related to higher numbers of the usual types of bacteria found in the udder. It has not been possible to make a detailed differential study of the types of bacteria found in quarters producing normal milk and of those producing abnormal milk, hence the conclusion that the disturbance is due to an increased development in the udder flora over that found in the normal quarter is a purely tentative one. The factors that restrain the growth of the usual udder forms are not known. If these factors are decreased in effect, the udder flora would grow more freely. The growth of any type of organism in the udder may elicit a response, which alters the composition of the milk.

Observations do not indicate that the cause of the condition passes from animal to animal.

In some of the animals the abnormality in a quarter was present at the beginning of the lactation period and persisted throughout the period. In other cases the abnormality appeared at some time during the lactation period and continued to the end thereof, and in still other cases it disappeared.

One or more samples of milk which satisfied the criteria of abnormality used, namely, a pH value above 6.8, a chlorine value above 0.15 percent, and a catalase value in excess of 50 percent, were obtained from 23 of the 31 cows. The detailed study of the records indicates that 17 of the animals may be considered as normal; 14 as abnormal. The detailed data of some of the abnormal animals are presented.

The same condition, that is, abnormal milk in the absence of any definite bacterial cause, is being studied in a second group of first-lactation-period animals. The significance of the abnormality to the herd owner and to the milk consumer is discussed.

DISTRIBUTION OF HOG-CHOLERA VIRUS AMONG FRACTIONS OF VIRUS BLOOD[1]

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REVIEW OF LITERATURE

A few workers who have investigated the distribution of hog-cholera virus between cells and serum of virus blood have reported results which indicate that an important part of the virus is firmly associated with the cells. Yet no adequate comparison of the virulence of the two fractions has been reported.

Dinwiddie (*1*)[3] administered various fractions of virus blood to susceptible pigs in doses ranging from 0.5 to 5 cc of washed cells and from 1 to 10 cc of plasma or serum. He validly concluded that the virus is prominently associated with the corpuscular fraction. Because of the relative severity of the disease produced in the experimental animals, there was the additional suggestion that the cell fraction may have carried relatively more virus than the serum or plasma.

Similar results were obtained in experiments reported by Hutyra and Köves (*4*), in which 0.5 cc of unwashed cells, 0.5 cc of washed cells, and 0.5 cc of cell-free serum—all from virus blood—infected or killed 8, 6, and 4 pigs, respectively, out of groups of 10.

Roderick and Schalk (*7*) showed that virus blood cells may carry and retain an infective dose of virus after nine washings with salt solution, and that 2 cc of normal-pig blood cells mixed with 1 cc of virus blood serum may take up and retain, through eight washings, an infective dose of virus.

Duval (*2*), differing from other authors, stated that freshly washed virus blood cells failed to infect and that the virus was never intracellular.

Rucks and Murray (*8*) found that 1-cc doses of virus blood cells, washed 6, 8, or 16 times, were uniformly infective and, as judged by the severity of the disease produced, appeared to contain more hog-cholera virus than 2½-cc doses of the corresponding cell-free serum. The Berkefeld filtrate from hemolyzed washed virus blood cells appeared to be at least equal in infectivity to the unfiltered material.

In experiments carried out at the field station of the Biochemic Division at Ames, Iowa, subsequent to the experiments described herein, McBryde and Cole, as reported by the Chief of the Bureau of Animal Industry (*6, Rept. 1931, p. 24*), found virus blood cells to be

[1] Received for publication Sept. 4, 1936; issued Feb. 1, 1937. A brief statement of the most important results of the experiments herein reported has been published (*6, Rept. 1930, p. 27*).

[2] The author wishes to acknowledge the helpfulness of his late chief, M. Dorset, a friend and counsellor, under whose general instructions this work was carried out. He wishes also to express his appreciation of the assistance and advice of C. N. McBryde, in charge of the Bureau's field station at Ames, Iowa, who gave so generously of his time in arranging facilities for the work and in making the actual inoculations. To C. G. Cole, assistant veterinarian at that station, he is likewise indebted for the observations of temperatures and symptoms and for the bleeding and post-mortem examinations of the experimental animals.

[3] Reference is made by number (italics) to Literature Cited, p. 233.

infective after seven washings with physiological salt solution and also found that cells obtained from normal defibrinated swine blood were capable of adsorbing virus from virus blood serum in vitro.

In all this work no attempt seems to have been made to compare the minimal lethal or infective doses of the materials under study. That these were much smaller than the doses actually employed may be conjectured from the work of Roderick and Schalk (7), who found a minimum infective dose for virus blood serum of one-fiftieth cubic millimeter, and the work of McBryde (5), who found a minimum lethal dose for defibrinated blood of one three hundredth to one four-hundredth cubic millimeter. It thus appears that existing information on the relative virulence of cell and serum fractions of virus blood is based on observations of the relative severity or frequency of infection produced by thousands of times the minimum lethal dose of the respective fractions. In view of the well-known variation among pigs in natural susceptibility to hog cholera, the reliability of such indications may well be questioned.

EXPERIMENTAL PROCEDURE

In the following experiments conducted in 1929 at the Ames field station of the Bureau of Animal Industry, an attempt was made to compare the minimal infective doses of the cells, the serum, and certain other fractions of virus blood and to measure the extent to which blood cells of normal pigs can adsorb hog-cholera virus from infectious serum.

The virus blood employed in these experiments was defibrinated and strained through sterile gauze with aseptic technique but was otherwise untreated. It was obtained at the height of the disease by exsanguination of experimentally inoculated hogs that had shown symptoms and temperatures of acute hog cholera. The diagnosis of hog cholera was further confirmed, sometimes by ante-mortem examinations for leucopenia, and always by noting at post-mortem examination the presence of typical cholera lesions and the absence of complicating conditions. In some experiments a mixture of three or four equally satisfactory samples of virus blood, collected on the same day, was employed as experimental material.

In the fractionation of the virus blood and the preparation of dilutions, the aim throughout was to conduct all operations as quickly as possible and with aseptic technique and to hold in cold storage all materials not actually under manipulation. Although the experiments varied in respect to the fractions under study, in all cases an initial separation of the virus blood into cell and serum fractions was made by centrifuge as soon as possible after the collection of the sample, and, except in experiments 4 and 5, the cell fraction was immediately thereafter washed by centrifuge with 0.85-percent salt solution until all but insignificant traces of serum were removed.

Unless otherwise noted, all dilutions of the various fractions were prepared with 0.85 percent salt solution. Moreover, unless otherwise noted, the first dilution consisted in bringing the volume of the suspended or dissolved fraction to the volume of virus blood from which it was derived. Further dilutions were made serially with standardized volumetric apparatus in such fashion that the measurement of small volumes was avoided. Dilutions for inoculation were prepared to contain the material derived from the desired quantity of virus blood in a

5-cc dose. Care was exercised throughout to provide for the homogeneous mixing of intermediate and final dilutions before use.

All test pigs employed in these experiments were nonimmunized animals known to have been bred from susceptible sows on nearby farms that had been free from hog cholera for several years. With the exception of pig 94 in experiment 4 all pigs used in a single experiment were from the same herd. Inoculations were always subcutaneous, and the dose of experimental material was always 5 cc. A separate syringe was used for each fraction, the higher dilutions being injected first. In making the injections, glass Luer syringes were used to insure accuracy. The syringes were fitted with small needles to guard against leakage of the injected material from the needle puncture. After inoculation each pig was placed in a separate detached pen that had been previously disinfected and was kept there under observation for 2 to 3 weeks. Temperatures were taken no oftener than was required to obtain adequate data on the course of the experiment and with all precautions to avoid accidental infection of the experimental animals.

EXPERIMENT 1

In experiment 1 dilutions of virus blood and of the washed cells and the serum separated from the same blood were injected into susceptible pigs in doses suggested by the work of Roderick and Schalk *(7)* and that of McBryde *(5)*. The virus blood was derived from a single pig, and the time elapsing from the drawing of the blood to the injection of the dilutions was 2 days. As shown by table 1, the results were completely negative. However, in subsequent experiments after these pigs had been inoculated with larger doses of virus material, mostly in admixture with certain chemicals, all of them succumbed to hog cholera except no. 246, which recovered after a well-marked reaction. Whether, in experiment 1, the virus blood was deficient in virulence or the experimental animals in susceptibility is not clear, but the doses employed were below the level of infectivity.

TABLE 1.—*Results of inoculating susceptible pigs with minute quantities of blood taken from a pig affected with hog cholera, and of the serum and the washed cells derived from the blood*

Material injected	Volume of blood from which dose was derived [1]	Pig no.	Approximate weight of pig	Results
	Cubic millimeter		*Pounds*	
Whole blood	1/250	242	50	Animal remained well during 12 days of observation.
	1/1250	243	50	Do.
Serum	1/125	244	50	Do.
	1/50	245	50	Do.
Washed cells	1/250	246	60	Do.
	1/250	247	60	Do.

[1] Diluted in all cases to a 5-cc dose.

EXPERIMENT 2

In experiment 2, dilutions of virus blood and of various fractions of this blood—serum, cells, erythrocytes only, and hemolyzed cells with stroma removed—were injected into susceptible pigs. In view of the results obtained in experiment 1, the maximum doses used in experiment 2 were larger.

The virus blood for this experiment was obtained from a single pig which, in addition to the usually observed criteria of hog cholera, had shown ante-mortem leucocyte counts of from 4,700 to 6,000 per cubic millimeter. The defibrinated sample contained 5,892,000 erythrocytes per cubic millimeter.

The virus blood, washed cells, serum, and dilutions thereof were prepared as already described, except that the wash fluid from the cell fraction was combined with the serum fraction, and the concentration of the initial cell dilutions was calculated from a blood count. Not more than 0.01 cc of serum could have remained with the washed cells derived from 100 cc of virus blood. The temperature in the centrifuge cups at the end of the washing process had risen to 34° C. The dilutions of these fractions were injected into susceptible pigs the day after the virus blood was collected.

On the second day after the collection of the virus blood, the erythrocyte fraction was prepared by removing the white cells from the initial dilution of the washed cells by a method described by Hornby (*3*), which consisted in passing the cell suspension through a 20-cm column of tightly packed cotton in an 8-mm diameter glass tube, the operation being carried out at a temperature of about 55° F. After 2 hours, about 7 cc of the material had passed, which was found by the usual technique to contain 2,644,000 erythrocytes per cubic millimeters. A white-cell count, made on a 1+1 instead of the usual 1+9 dilution, showed no white cells. After remaining overnight in cold storage, the well-mixed "filtrate" was further diluted with 0.85-percent salt solution to give suspensions containing in 5-cc portions the desired number of erythrocytes. Three days elapsed between the collection of the virus blood and the injection of these dilutions into susceptible pigs.

In making the hemolyzed-cell dilutions, immediately after removal of the leucocytes, 1 cc of the original erythrocyte suspension was added to 44 cc of distilled water, and the mixture was allowed to stand overnight in cold storage. On the following morning 15 cc of the hemolysate was centrifuged until no stroma could be found in the supernatant by microscopic examination after use of aniline methyl violet stain. Dilutions of this material were prepared with 0.85-percent salt solution to correspond in strength to the final dilutions of the erythrocyte suspension. These dilutions were also injected into susceptible animals 3 days after collection of the virus blood.

To test the susceptibility of the pigs, one animal was inoculated with 5 cc of stock virus of known virulence. To test the virulence of the experimental virus blood, another animal was inoculated with 5 cc of this blood.

The results of this experiment are given in table 2.

TABLE 2.—*Results of inoculating susceptible pigs with minute quantities of blood taken from a pig affected with hog cholera, and of the serum, the washed cells, the erythrocytes only, and the stroma-free hemolysate prepared from this blood*

Material injected	Volume of blood from which dose was derived [1]	Pig no.	Approximate weight of pig	Results
	Cubic millimeters		*Pounds*	
Whole blood	1/500	20	50	Normal to twentieth day.
	1/100	21	55	Do.
	1/50	22	50	Suspicious temperatures sixth and ninth days; slight symptoms eighth to thirty-sixth days; apparently chronic hog cholera.
Washed cells	1/480	26	55	Normal to twentieth day.
	1/93	27	55	Suspicious symptoms without temperature eleventh, twelfth, and thirteenth days; normal to twentieth day.
	1/47	28	55	Suspicious symptoms without temperature eleventh, twelfth, and thirteenth days; normal to twentieth day; cholera with recovery from test injection of virus twenty-second day.
Erythrocytes	1/240	50	55	Acute cholera beginning seventeenth day; confirmed post mortem twenty-third day.
	1/80	51	60	Acute cholera beginning seventh day; confirmed post mortem fourteenth day.
	1/40	52	60	Acute cholera beginning ninth day; confirmed post mortem eighteenth day.
Hemolysate	1/240	53	60	Normal to seventeenth day.
	1/80	54	50	Do.
	1/40	55	50	Suspicious temperature and symptoms eleventh day only; decided reaction with recovery in 7 days from test injection of virus twenty-fourth day.
Serum	1/100	23	55	Normal to twentieth day.
	1/50	24	55	Do.
	1/10	25	55	Symptoms eighth to twelfth day; temperature ninth day; recovered immune.
Stock virus (control)	5,000	30	50	Acute cholera beginning third day; animal killed seventh day.
Whole blood (control)	5,000	29	50	Do.

[1] Diluted in all cases to a 5-cc dose.

The evidence obtained indicates that the serum fraction of this virus blood carried probably not more than one-fifth of the infectivity of the whole blood. Beyond this, it is inadvisable to draw conclusions from this experiment. Evidently, in some instances border-line doses were being used, the infectiousness of which undoubtedly depended in part on the degree of resistance of the inoculated animals. This circumstance may account for the fact that the erythrocyte fraction appeared to be so much more virulent than the whole blood or the washed-cell fraction, both of which contained all of the erythrocytes, or than the hemolysate, which contained the erythrocytes less stroma. Otherwise one would need to postulate that the leucocytes in the mixed-cell fraction and the serum or leucocytes in the whole blood are endowed with antivirulent properties and that a considerable quantity of infective material, associated with the stroma, was removed in the preparation of the hemolysate.

EXPERIMENT 3

Experiment 3 was designed to supplement experiment 2 because of the somewhat ambiguous and contradictory results obtained in that experiment. Five dilutions each of whole virus blood, of washed virus blood cells, and of the filtered hemolysate prepared from the washed cells were injected into susceptible pigs. All inoculations were made on the third day after the virus blood was collected. For control purposes a single pig, no. 75, was inoculated with 2 cc of the undiluted virus blood. The original virus blood represented the mixed blood of three cholera-sick hogs and contained 6,252,000 erythrocytes and 2,200 leucocytes per cubic millimeter. The cell fraction was separated and washed, and dilutions of whole blood and of washed cells were prepared as already described. It was calculated that at the end of washing, the cells from 100 cc of the virus blood contained not more than 0.0001 cc of serum. One cubic centimeter of washed-cell suspension, containing the cells from 1 cc of blood, was brought to a volume of 50 cc with distilled water and held overnight in cold storage, after which the hemolysate was sharply centrifuged and the supernatant was passed through a sterile Berkefeld filter. This filtrate was used for the preparation of the required dilutions. The pertinent data for this experiment are summarized in table 3.

TABLE 3.—*Results of inoculating susceptible pigs with minute quantities of mixed blood from 3 pigs affected with hog cholera, and of the washed cells and the stroma-free hemolysate prepared from this blood*

Material injected	Volume of blood from which dose was derived [1]	Pig no.	Approximate weight of pig	Results
	Cubic millimeters		*Pounds*	
Whole blood	1/500	60	70	Normal to fourteenth day.
	1/200	61	60	105° F. seventh day; otherwise normal to fourteenth day.
	1/100	62	50	Normal to fourteenth day.
	1/50	63	50	Do.
	1/25	64	50	Temperature 104° seventh day; suspicious symptoms seventh to twelfth days.
Washed cells	1/500	65	70	Temperature 104° fourth day and 104.4° twelfth day; suspicious symptoms ninth to fourteenth days.
	1/200	66	60	Suspicious symptoms thirteenth day.
	1/100	67	65	Suspicious symptoms seventh day; temperature 104° fourth day.
	1/50	68	60	Normal to fourteenth day.
	1/25	69	60	Do.
Hemolysate	1/500	70	75	Do.
	1/200	71	60	Suspicious symptoms eighth to eleventh days.
	1/100	72	70	Normal to fourteenth day.
	1/50	73	70	Suspicious symptoms eleventh to twelfth days.
	1/25	74	60	Normal to fourteenth day.
Whole blood (control)	2,000	75	65	Acute cholera, initial symptoms fourth day; killed eighteenth day.

[1] The dose for the control pig was 2 cc of undiluted blood. For all other pigs, dilutions were made to a 5-cc dose.

Except for the control animal, no frank case of hog cholera developed in any of the experimental animals during the 14-day observation period, though five scattered temperature readings of 104° to 105° F.

were obtained, and occasional suspicious symptoms were noted in a few of the animals. This is surprising in view of results obtained in other experiments in which smaller quantities of virus blood or its fractions were infective. Although the control pig developed acute cholera, its survival for 18 days suggested that the virus used was not of maximum virulence. This experiment illustrates the uncertainties with which this type of experiment is beset, owing to the impossibility of controlling the potency of virus and the susceptibility of pigs.

At the end of the observation period, before the pigs were sent to quarantine pasture, all of them except no. 75 received simultaneous immunization. Pig 66 was given this treatment on the sixteenth day after inoculation and all other pigs on the fourteenth day. Pig 64 was found dead the morning after receiving the immunizing treatment; pig 65 remained in poor condition; all other pigs remained normal.

EXPERIMENT 4

In experiment 4 dilutions from four fractions of virus blood—serum, unwashed cells, erythrocytes only, and erythrocyte stroma—were injected into susceptible pigs in the quantities indicated in table 4. For control purposes 4 cc of the original virus blood was injected into a single pig.

The mixed blood from four cholera-sick hogs, with ante-mortem leucocyte counts of 5,900, 9,700, 18,600 to 9,300, and 9,100 to 7,300, respectively, per cubic millimeter, constituted the virus blood used in this experiment.

The serum was separated from the virus blood in the customary manner but showed decidedly more hemolysis than usual. Through oversight the serum dilutions were made without first diluting the serum to the volume of blood from which it was derived. However, on the calculation that 2 cc of serum was derived from at least 3 cc of defibrinated blood, the doses actually employed were derived from not less than the quantities of blood indicated in table 4. These doses were injected into susceptible pigs the day after the virus blood was collected.

The cells were sedimented from the virus blood by sharp centrifuging, and the serum was removed as completely as possible. The usual washing was omitted. After replacement of the serum with an equal quantity of salt solution and after mixing, a cell enumeration showed 6,036,000 erythrocytes per cubic millimeter. Dilutions of this suspension were prepared in the usual manner and were injected into susceptible pigs the day after the collection of the virus blood.

By the technique employed in experiment 2 the white cells were removed from a portion of the unwashed cell suspension. The recovered erythrocyte suspension contained 3,144,000 erythrocytes and no leucocytes per cubic millimeter. On the basis of the cell count, dilutions of this material were made to contain in 5 cc portions the erythrocytes from the desired volumes of the original virus blood. These dilutions were injected into susceptible pigs the day after the virus blood was collected.

In obtaining the stroma, 2 cc of a freshly prepared erythrocyte "filtrate" of unmeasured cell content was laked with 13 cc of water and a few drops of ether. The following day the hemolysate was centrifuged sharply for the recovery of the stroma. The recovered sedi-

ment was resuspended in distilled water and resedimented three successive times, the final sediment of about 0.4 cc containing the soluble cell material from not more than one two-hundred-and-fifty-thousandths of a cubic centimeter of blood. This material was suspended in 10 cc of water, and 5 cc of the suspension was injected into a susceptible pig on the second day after the virus blood was collected. On the basis of the cell count made on the first erythrocyte filtrate, it seems fair to conclude that the injected dose of stroma was derived from not more than 0.5 cc of the original virus blood.

The pertinent data relating to the inoculated pigs are summarized in table 4.

TABLE 4.—*Results of inoculating susceptible pigs with minute quantities of serum, mixed cells, and erythrocytes only, and with a much larger quantity of stroma, all prepared from mixed blood taken from 4 cholera-sick hogs*

Material injected	Volume of blood from which dose was derived [1]	Pig no.	Approximate weight of pig	Results
	Cubic millimeters		*Pounds*	
Serum	[2] 1/33	105	60	Normal to eighteenth day; (succumbed to cholera in subsequent experiment).
	[2] 1/17	106	60	Normal to eighteenth day; (subsequently, as pig 132 in experiment 6, developed chronic cholera when injected with minute quantity of virus material).
Mixed cells	1/200	101	60	Normal temperature; suspicious symptoms tenth to fourteenth days; fair recovery.
	1/100	102	60	Acute cholera beginning fifth day; killed moribund twenty-first day.
	1/50	103	60	Acute cholera beginning fourth day; killed for virus tenth day.
	1/25	104	60	Acute cholera beginning seventh day; improvement fourteenth to twenty-fourth days.
Erythrocytes	1/200	107	60	Normal to eighteenth day; (subsequently, as pig 129 in experiment 6, developed acute cholera when injected with minute quantity of virus material).
	1/100	108	60	Normal to eighteenth day; (succumbed to cholera in subsequent experiment).
	1/50	109	60	Normal to eighteenth day; (developed chronic cholera in subsequent experiment).
	1/25	110	60	Normal to eighteenth day; (subsequently, as pig 127 in experiment 6, developed acute cholera when injected with minute quantity of virus material).
Stroma	500	111	60	Acute cholera beginning third day; killed moribund eleventh day.
Whole blood (control)	4,000	94	35	Do.

[1] The dose for the control pig was 4 cc of undiluted blood. For all other pigs, dilutions were made to a 5-cc dose.

[2] This represents the approximate volume of blood.

In this experiment the virus was preponderantly associated with the cellular fraction. The results obtained for the erythrocytes alone appear inconsistent with those obtained in experiment 2, in which the erythrocytes seemed to show greater virulence in corresponding doses than the mixed cells. The reason for this discrepancy is not clear, though the present results appear to be more reasonable.

EXPERIMENT 5

Experiment 5 was designed to provide additional information on the relative infectivity of the mixed cells and erythrocytes only, and to test the infectivity of smaller doses of the stroma fraction than those employed in experiment 4. The virus blood employed in this experiment was the mixed blood from three cholera-sick pigs. The serum, unwashed cells, erythrocytes, and stroma were severally separated from the virus-blood sample and suitably diluted for inoculation.

In the erythrocyte fraction, complete removal of leucocytes was not achieved, although their number relative to that of the red cells was reduced to less than one-third of the original number. In the preparation of the stroma fraction, ether was used as in experiment 4; and in addition carbon dioxide at alveolar tension was bubbled through the laked erythrocytes prior to sedimentation of the stroma in the centrifuge. Washing of the stroma sediment was continued until the stroma from 2,500 cubic millimeters of blood contained the soluble material from not more than 0.02 cubic millimeter of blood.

In all cases, the prepared dilutions were injected into susceptible pigs the day after the original virus blood was collected. The volume of blood from which these doses were derived, together with the pertinent data concerning the experimental animals, are included in table 5.

TABLE 5.—*Results of inoculating susceptible pigs with minute quantities of serum, mixed cells, erythrocytes only, and washed stroma, all prepared from mixed blood taken from 3 cholera-sick hogs*

Material injected	Volume of blood from which dose was derived [1]	Pig no.	Approximate weight of pig	Results
	Cubic millimeters		*Pounds*	
Serum	1/25	116	100	Acute cholera beginning sixth day; recovery by twenty-second day.
Mixed cells	1/100	117	100	Acute cholera beginning fourth day; killed moribund eighteenth day.
	1/50	118	100	Acute cholera beginning fourth day; died eighteenth day.
Erythrocytes	1/50	119	90	Acute cholera beginning fourth day; recovery by twenty-second day.
	1/25	120	100	Acute cholera beginning sixth day; temporary improvement twentieth day and after; placed on quarantine pasture twenty-second day; dead with lesions thirty-ninth day.
Stroma	1/25	121	100	Acute cholera beginning sixth day; killed moribund twenty-ninth day.
	2	122	100	Acute cholera beginning fourth day; killed for virus eighth day.
	100	123	100	Acute cholera beginning fourth day; recovery by twenty-second day.
Whole blood	5,000	124	65	Acute cholera beginning seventh day; killed moribund twentieth day.

[1] Diluted in all cases to a 5-cc dose.

This experiment affords no quantitative measure of the minimum infective dose of any of the virus fractions studied, and therefore no comparison of the relative infectivity of the several fractions can be made. It is evident, however, that each fraction, in the smallest dose employed, contained at least one minimum infective dose of virus. In the case of the stroma an exceptionally high concentration of virus relative to the accompanying inert matter seems to have been achieved.

EXPERIMENT 6

Experiment 6 was intended to demonstrate (1) possible adsorption of hog-cholera virus from virus blood serum by the cells of normal-pig blood and (2) a possible resultant decrease in the infectivity of the virus serum.

The virus blood for this experiment was obtained from pig 122 that had developed hog cholera in experiment 5. On the same day a sample of normal blood was obtained from the tail of a healthy pig. Both blood samples were defibrinated, and 15-cc portions of each were promptly centrifuged for the separation of cells and serum, 6.8 cc of virus blood cells and 8.2 cc of virus blood serum being obtained from the virus blood. The virus cells were rejected. The virus blood serum was recentrifuged, separated from a small sediment, and mixed with 6.8 cc of the cell sediment obtained from the normal blood. This mixture was incubated at 34° to 37° C. for 4 hours, when the cells and serum were again separated by centrifuge. From the serum thus recovered, augmented by the first washings of the cells, dilutions were prepared, doses of which were injected subcutaneously into two susceptible pigs on the day after the virus blood was obtained.

The normal cells that had been exposed to virus blood serum were repeatedly washed by centrifuge with 0.85-percent salt solution until they retained less than 0.002 cc of the serum and were used in the preparation of appropriate dilutions. These dilutions were injected into two susceptible pigs the day after the experimental materials were collected.

From a second preparation of virus blood serum derived from the same blood, dilutions were prepared directly. Because of failure to bring the serum to the volume of the original blood at the beginning of the diluting process, the dilutions finally obtained contained in 5-cc portions the untreated virus blood serum from 0.073 and 0.037 cubic millimeter, respectively, of the original virus blood. These doses were injected subcutaneously into two susceptible pigs the day after the virus blood was collected.

The important data relating to the inoculated pigs are summarized in table 6.

TABLE 6.—*Results of inoculating susceptible pigs with minute quantities of untreated virus serum, of the same virus serum after exposure to normal-pig blood cells, and of the normal-pig blood cells after exposure to the virus serum*

Material injected	Volume of blood from which virus was derived[1]	Pig no.	Approximate weight of pig	Results
	Cubic millimeters		*Pounds*	
Untreated virus blood serum	1/17	127	85	Acute cholera fourth day; killed moribund nineteenth day.
	3/17	128	85	Acute cholera fourth day; died eighteenth day.
Virus blood serum exposed to blood cells of normal pig	1/25	129	85	Acute cholera fourth day; killed moribund eighteenth day.
	3/25	130	90	Normal throughout.
Blood cells of normal pig exposed to virus blood serum	1/25	131	80	Acute cholera fifth day; killed moribund twenty-second day.
	3/25	132	95	Acute cholera fourth day; became chronic; killed worthless thirty-eighth day.

[1] Diluted in all cases to 5-cc dose.

The results of this experiment do not permit a mathematically exact computation of the percentage of virus adsorbed from virus blood serum. However, on the basis of the evidence obtained in the preceding experiments and that obtained by Roderick and Schalk (7), it seems probable that not much more than two minimum infective doses could have been contained in one twenty-fifth of a cubic millimeter of the virus blood serum employed in this experiment. Yet from this quantity of virus serum, about one-thirtieth of a cubic millimeter of normal cells adsorbed at least one minimum infective dose. Presumably about one-half of the virus had been adsorbed upon the normal blood cells.

DISCUSSION

To obtain precise results with unstandardized materials is not possible, and in the nature of the case pigs cannot be standardized for susceptibility nor virus for potency in experiments of this kind. It is, therefore, not surprising that the results reported in this paper show irregularities and, at times, apparent conflicts. That a complete picture of these difficulties may be presented, all results, both negative and positive, have been included.

From the results obtained on pigs nos. 50 and 51 in experiment 2, it is clear that the erythrocytes from 1 cubic millimeter of virulent blood contained at least 80 and presumably at least 240 minimal infective doses of virus, though because of the delayed appearance of symptoms in pig no. 50 some degree of uncertainty may attach to the validity of the latter figure. Similarly, from the results obtained on pigs nos. 24 and 25 in the same experiment, it is clear that the serum from the same volume of virus blood contained more than 10 and less than 50 minimal infective doses. It thus appears that the erythrocyte fraction of this blood contained at least 1.6 times (80÷50), possibly 24 times (240÷10), as much virus as did the serum fraction. Again in experiment 4, the serum from 1 cubic millimeter of virulent blood contained less than 17 minimal infective doses, whereas the washed cells from the same volume of blood contained 100 or more minimal infective doses. Thus, in this experiment the cell fraction from virulent blood appeared to contain a minimum of about six times as much virus as did the corresponding serum fraction. Without attempting to generalize on the basis of such limited data, it is at least evident that in these experiments most of the infectivity of the virus blood was associated with the cell fraction.

In respect to the comparative virulence of the total cell fractions and of the erythrocyte fractions, experiments 2 and 4 are in apparent disagreement. The results on the mixed-cell fraction in experiment 2, however, are the less convincing, being difficultly reconcilable with those on the erythrocytes only in the same experiment, and being in conflict with those obtained for the mixed-cell fractions in experiments 4 and 5. The nonvirulence of the erythrocyte doses in experiment 4 is likewise in conflict with the high virulence of the erythrocyte fractions in experiments 2 and 5, and of the stroma fraction in experiment 5. The preponderance of evidence, in any case, is to the effect that there is little if any difference in infectivity between the mixed cells and the erythrocytes only and that the leucocytes probably play no important part as carriers of virus.

A direct study of the infectivity of the erythrocyte stroma from virus blood apparently has not previously been made, although Rucks and Murray (*8*) showed that the stroma-free hemolysate from 1 cc of virus blood cells was apparently equal in infectivity to 1 cc of the original cells. Considerable differences in infectivity, however, might be overlooked through the use of such large doses. Actually, experiment 5 of this paper, without indicating the limit of infectivity of the stroma, showed that the washed stroma from one twenty-fifth of a cubic millimeter of virus blood was decidedly infectious. Undoubtedly the stroma carried a very significant part of the virus in the original blood. On the basis of experience in this laboratory, it seems fair to conclude that the stroma fraction of experiment 5 contained not more than 4 percent of the total solids of the original blood. It seems fairly certain, therefore, that per unit of total solids, the amount of virus in this fraction was equivalent to that in a virus blood with a minimum infective dose of one six-hundred-and-twenty-fifth of a cubic millimeter. Apparently the stroma fraction constituted a more concentrated source of virus than the whole virus blood.

Throughout these experiments, whenever a valid comparison could be drawn between fractions prepared by centrifuge, the sediment has appeared to be more virulent than the supernatant. Presumably, the virus was associated with the sedimented material in the original blood. Certainly, it has hitherto been assumed that hog-cholera virus, as such, is not sedimented at the centrifuge speeds here employed. Yet, so far as the author is aware, this assumption is not based on critical experimental evidence. An adequate experimental treatment of this question would be desirable.

SUMMARY

Experiments were conducted in 1929 by the Bureau of Animal Industry at its field station, Ames, Iowa, in an attempt to compare the minimal infective doses of the cells, serum, and certain other fractions of virus blood and to measure the extent to which blood cells of normal pigs can adsorb hog-cholera virus from infectious serum. The virus blood was obtained from experimentally inoculated hogs that had shown symptoms and temperatures of acute hog cholera. The test pigs used were nonimmunized animals bred from susceptible sows on nearby farms that had been free from hog cholera for several years. The following results were obtained:

Most of the virus of hog-cholera blood was associated with the cellular elements.

Probably no important part of the whole quantity of virus in hog-cholera blood was associated with the leucocytes.

The erythrocyte stroma of hog-cholera blood carried an important part of the virus in a relatively high concentration.

Blood cells of normal pigs, when mixed with virus serum, readily and firmly adsorbed a considerable part of the hog-cholera virus that the serum contained.

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A STATISTICAL STUDY OF SQUAB PRODUCTION IN THE WHITE KING BREED[1]

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INTRODUCTION

The production of squabs has never received very much attention in the field of agricultural research. Pigeons have been used for experimental purposes to determine nutritive requirements for maintenance and reproduction, but so far as the writer has been able to determine there are no records of the effect of various biological factors, such as egg production, fertility, hatchability, livability, squab weight, and number of squabs matured, upon total weight of squabs produced in a year by a pair of mated pigeons. The aim of the study herein reported was to establish these relationships in the White King breed.

MATERIALS AND METHODS

The records of the first 4 years, 1931 to 1934, of the New Jersey State pigeon breeding test were used as the source of the data. Only the White King records were used as this was the most popular breed and offered the most material. The pairs used in the study were entered by pigeon breeders from their home flocks. The birds were sent to the test plant on October 1 of each year and kept there for 51 weeks. Only the records of those that lived for the full period were used. Each entry consisted of five or six pairs, and was given an individual pen and fly. The feeding and management were uniform for all pens during any one year, although there was some change in the feeding from year to year.

In computing egg production the number of eggs laid during the year was used. Fertility and hatchability were based upon the number of eggs observed, after deducting from the total laid those eggs that were in the nest at the close of the year. Livability of squabs was based upon the total number of squabs officially weighed after deducting those that were in the nest at the close of the year. All squabs 21 days old or older were weighed at the close of the year; during the year the weights were taken when the squabs were in marketable condition, that is, from 25 to 28 days of age.

STATISTICAL ANALYSES

Figure 1 shows the distribution of the 138 pairs of pigeons used. Judging by the comparison of the observed and normal curves, and also by a P value of 0.713 secured by the χ^2 test, it would seem that the population was representative of the breed under the conditions specified.

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The mean annual production of 225.90±3.57 ounces of squabs (table 1) is of particular interest because of its similarity to the mean annual egg production of White Leghorn pullets that lived a year in the Hunterdon County egg-laying test of 1933–34, the latter figure being 216.26±1.63 eggs (unpublished data). The coefficient of variation in the ounces of squabs produced was 27.50±1.22 percent as compared with a coefficient of 23.49±0.58 percent in egg production

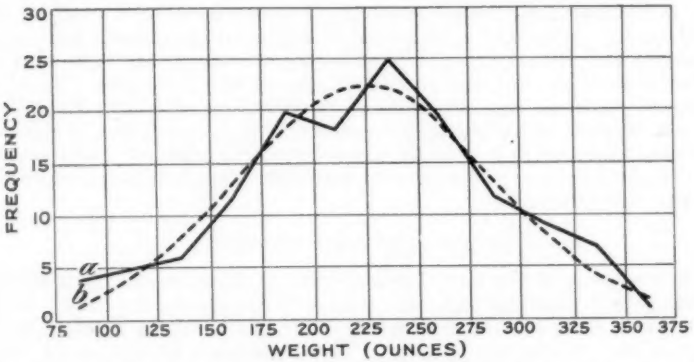

FIGURE 1.—Distribution of pairs of White King pigeons according to ounces of squabs produced annually: *a*, Observed frequencies; *b*, normal curve.

of the White Leghorn pullets. The two species show a close similarity in the production of their marketable products when one is measured in ounces and the other in eggs.

TABLE 1.—*Statistical measurements of annual squab production per pair of White King pigeons*

Item	Mean	Standard deviation	Coefficient of variation
Total production (live weight)....ounces..	225.90±3.57	62.13±2.52	27.50±1.22
Total squabs....number..	11.49± .18	3.10± .13	26.98±1.89
Fertility....percent..	85.21± .61	10.67± .43	12.52± .52
Livability of squabs....do....	89.70± .60	10.40± .42	11.60± .48
Eggs laid....number..	16.85± .17	2.98± .12	17.69± .74
Hatchability of fertile eggs....percent..	94.16± .32	5.60± .23	5.95± .25
Average squab weight....ounces..	19.62± .10	1.81± .07	9.22± .38

The relative effect of the factors studied upon the total number of ounces of squabs produced in a year can be secured by observing the coefficients of correlation shown in the following tabulation. A very high relationship existed between the number of squabs and the total weight of squabs matured by a pair of pigeons, the correlation coefficient being 0.9512±0.0055. The fertility of the eggs was next in importance with livability of squabs almost of equal value in its relationship to total weight of mature squabs.

Coefficients of correlation between total weight of squabs produced annually and certain observed factors

	Coefficient of correlation
Total number of squabs	0.9512±0.0055
Percent fertility of eggs	.5874± .0476
Percent livability of squabs	.5002± .0431
Egg production per pair	.2877± .0526
Percent hatchability of fertile eggs	.1470± .0562
Average squab weight	.0010± .0568

Egg production and hatchability both showed a positive correlation but not so high a one as the other factors mentioned. Average squab weight did not bear any relation to total weight of squabs produced, the coefficient of correlation being 0.0010 ± 0.0568.

SUMMARY

Data on the total number of eggs laid annually, the percent fertility of the eggs, the percent hatchability of the fertile eggs, the percent livability of the squabs, the total number of squabs reared, the average squab weight, and the total ounces of squabs produced annually were secured from 138 pairs of White King pigeons entered in the New Jersey State pigeon breeding tests. Statistical measurements of each factor were obtained, as were also the coefficients of correlation respectively between egg production, fertility, hatchability, livability, average squab weight, total number of squabs, and total ounces of squabs produced annually.

The mean annual weight of squabs per pair of pigeons that survived a year was 225.90 ± 3.57 ounces, a figure comparable to the mean annual egg production of White Leghorn pullets that survived a year in the Hunterdon County egg-laying test, 216.26 ± 1.63 eggs.

The highest correlation coefficient, 0.9512 ± 0.0055, was between the total number and total weight of squabs produced annually. Percentage fertility of eggs showed the next highest relationship to total weight of squabs produced, the correlation coefficient being 0.5874 ± 0.0476, while the average weight of squabs per pair of pigeons showed the least, which was indicated by a correlation coefficient of 0.0010 ± 0.0568.

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